Pneumococcal vaccination in inflammatory rheumatic disease. Antibody response and protection against infections.

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Pneumococcal vaccination in inflammatory rheumatic disease

Antibody response and protection against infections

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Pneumococcal vaccination in inflammatory rheumatic disease

Antibody response and protection against infections

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UNIVERSITY

DOCTORAL DISSERTATION

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Abstract

Objectives: Patients with inflammatory rheumatic diseases (IRDs) have higher risk of serious infections than healthy individuals due to their illness, comorbidities and treatments. *Streptococcus pneumoniae* is responsible for 30-50% of community aquired pneumonia in Europe.The proportion of patients with IRD receiving vaccination against pneumococci is still estimated to be suboptimal. The aims of this project was to investigate whether pneumococcal conjugated vaccination of patients with IRD leads to an antibody response adequate enough to protect against infections, and what treatments and/or other factors that influences the immunological and clinical response to vaccination.

Methods: Patients suffering from different forms of arthritis or systemic lupus erythematosus with different ongoing antirheumatic treatments were vaccinated with pneumococcal vaccine; 7- or 13-valent. Antibody response was analyzed using several laboratory methods; standard ELISA, multiplex fluorescent microsphere immunoassay and Opsonophagocytic assay. Skåne Healthcare Register was searched for ICD-codes corresponding to pneumococcal infections. Circulating plasmablasts from RA patients with or without methotrexate were isolated using ELISPOT.

Results: Arthritis patients who received 7-valent conjugated pneumococcal vaccine had a relative risk reduction of being diagnosed with a pneumococcal infection up to 4 years after the injection. Antibody levels of approximately 1 µg/ml or above was associated with reduced risk of infection. Higher age and oral prednisolone treatment at vaccination predicted an elevated risk of serious infection after vaccination. Patients with rheumatoid arthritis with methotrexate treatment presented lower antibody levels but equal numbers of vaccine-specific antibody-producing plasmablasts in comparison with patients without methotrexate. SLE patients on belimumab in addition to standard treatment did not show impaired antibody response of 12 measured serotype specific antibodies, compared to SLE patients on standard of care treatment.

Conclusion. Patients with arthritis or SLE have in most cases an adequate antibody response after conjugated pneumococcal vaccination, although reduced in comparison to healthy controls. There is a correlation between reduced antibody response after vaccination and higher risk of subsequent serious pneumococcal infections. Vaccination leads to a relative risk reduction of pneumococcal infection. Belimumab does not seem to affect antibody response. The antibody response among patients on methotrexate is reduced, but this does not seem to be caused by a reduced number of antibody secreting cells.

Key words: inflammatory rheumatic disease, pneumococci, conjugated vaccine, antibody response

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Content

List of papers 8
Abbreviations 9
Populärvetenskaplig sammanfattning 11
Allgemein wissenschaftliche Zusammenfassung 14
Introduction 17
Rheumatoid arthritis 19
Etiology and pathophysiology 19
Clinical features 20
Extra-articular disease, comorbidities and mortality 21
Infections in RA 21
Spondyloarthritis 23
Pathogenesis 23
Clinical features 24
Infections in spondyloarthritis 25
Systemic lupus erythematosus (SLE) 26
Etiology and pathophysiology 26
Clinical features 27
Comorbidities and mortality in SLE 28
Infections in SLE 29
Antirheumatic treatment 30
Streptococcus pneumoniae 33

Pneumococcal vaccines 34
Polysaccharide pneumococcal vaccine (PPV) 35
Conjugated pneumococcal vaccine (PCV) 35
Recommendations of pneumococcal vaccinations 36
Vaccine effectiveness 37
Antibody response 37
Aims of the present investigation 39
Patients and methods 40
Skåne Healthcare Register 41
Enzyme-linked immunosorbent assay (ELISA) 42
Opsonophagocytic Assay (OPA) 42
Enzyme-linked immunospot (ELISPOT) 43
Multiplex fluorescent microsphere immunoassay (MFMI / Luminex) 43
Statistical methods 44
Results and Discussion 46
Paper I: 46
Paper II: 51
Paper III: 54
Paper IV: 57
Conclusions 61
Future perspectives 62
Tack 65
References 67

List of papers

This thesis is based on the following papers, which will be referred to by their Roman numerals in the text.

I. **J Nagel**, P Geborek, T Saxne, G Jönsson, M Englund, I F Petersson, M C Kapetanovic. The risk of pneeumococcal infections after immunization with pneumococcal conjugate vaccine compared to non-vaccinated inflammatory arthritis patients. *Scandinavian Journal of Rheumatology 2015;44:271-279*

II. **J Nagel**, P Geborek, T Saxne, G Jönsson, M Englund, I F Petersson, J-Å Nilsson, L Truedsson, M C Kapetanovic. The association between antibody levels before and after 7-valent pneumococcal conjugate vaccine immunization and subsequent pneumococcal infection in chronic arthritis patients. *Arthritis Research and Therapy 2015; 17:124*

III. **J Nagel**, T Saxne, P Geborek, AA Bengtsson, S Jacobsen, C Svaerke Joergensen, J-Å Nilsson, L Skattum, A Jönsen, M C Kapetanovic. Treatment with belimumab in systemic lupus erythematosus does not impair antibody response to 13-valent pneumococcal conjugate vaccine. *Lupus 2017; 26:1072-1081*

IV. M C Kapetanovic, **J Nagel**, I Nordström, T Saxne, P Geborek, A Rudin. Methotrexate reduces vaccine-specific immunoglobulin levels but not numbers of circulating antibody-producing B cells in rheumatoid arhritis after vaccination with a conjugate pneumococcal vaccine. *Vaccine 2017; 35: 903-908*

Abbreviations

ACIP	Advisory Commity on Immunization Practices
ACPA	anti–citrullinated protein antibody
ANA	antinuclear antibody
APC	antigen presenting cell
APS	antiphospholipid syndrome
ARR	absolute risk ratio
AS	Ankylosing Spondylitis
ASC	antibody secreting cell
AZA	azathioprine
BlyS	B lymphocyte stimulator
CAP	community aquired pneumonia
CD	cluster of differentiation
CDC	Centers for Disease Control and Prevention
COX	cyclooxygenase
COMORA	comorbidities in Rheumatoid Arthritis
CRP	C-reactive protein
CSF	cerebrospinal fluid
CVD	cardiovascular disease
DAS28	disease activity score with 28 joint
DMARD	disease modifying anti rheumatic drug
DNA	deoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay
ELISPOT	enzyme-linked immunospot
ENA	extractable nuclear antigens
FITC	fluorescent isothiocyantate
GMC	geometric mean concentration
GML	geometric mean level
HAQ	health assessment questinnaire
HCQ	hydroxychloroquine
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
IBD	inflammatory bowel disease
IgA/IgG/IgM	immunoglobulines of isotypes A, G or M
IL	interleukin
ILD	interstitial lung disease
IPD	invasive pneumococcal disease
IRD	inflammatory rheumatic disease
IRS	inflammatorisk reumatisk sjukdom
MFMI	multiplex fluorescent microsphere immunoassay
MHC	major histocompability complex
MTX	methotrexate

NNT	number needed to treat
NVT	non vaccinetype
OPA	opsonophagocytic assay
PBMC	peripheral blood mononuclear cell
PCV	pneumococcal conjugate vaccine
PD	pneumococcal disease
PE	phycoerythrin
PPV	positive predictive value
RA	rheumatoid arthritis
RF	reumatioid factor
ROC	receiver operating characteristic
RR	relative risk
RRR	ratio of relative risk
SHR	Skåne health care register
SLE	systemic lupus erytematosus
SLEDAI	systemic lupus erythematosus disease activity index
SpA	spondyloarthritis
SSc	systemic sclerosis
SSZ	sulfasalazine
TNF	tumor necrosis factor
VAS	visual analogue scale
VT	vaccinetype
WHO	World Health Organisation

Populärvetenskaplig sammanfattning

Reumatoid artrit (RA), spondylartrit (SpA) och systemisk lupus erythematosus (SLE) är exempel på tillstånd som gemensamt kallas inflammatoriska reumatiska sjukdomar (IRS). Genetiska varianter och miljöfaktorer som till exempel ämnen i cigarettrök och bakterier i munhåla och tarm har visat sig spela roll för sannolikheten att utveckla dessa sjukdomar. De utlösande orsakerna är dock ännu inte helt klarlagda. Gemensamt för IRS är avvikelser i immunförsvaret som resulterar i att olika organ drabbas av inflammation, vilket kan ge kroniska symptom och vara skadligt för hela kroppen.

Förutom själva sjukdomen drabbas dessa individer ofta av en rad följdverkningar och ökade risker för annan sjuklighet som till exempel tumörer, hjärt-kärlsjukdomar och infektioner. I takt med utvecklingen av mer effektiva antireumatiska läkemedel de senaste decennierna har det allmänna måendet vid IRS förbättrats, de kroniska skadorna på till exempel leder minskat och livslängden ökat. Dock bidrar behandlingarna till en ytterligare ökad risk för infektioner jämfört med friska individer. Fallrapporter om nyinsjuknande och eller förvärring av reumatisk sjukdom i samband med vaccinationer mot infektionssjukdomar har förekommit och bidragit till en avvaktande attityd gentemot vaccinationer hos både patienter med IRS och deras behandlande läkare. Större kontrollerade studier har dock inte kunnat bekräfta någon riskökning för till exempel nyinsjuknande i RA vid vaccination, och dessa patienter rekommenderas både i Sverige och i övriga länder sedan många år att ta emot flera sorters vaccinationer för att skydda sig.

Den här avhandlingen handlar om vaccination mot pneumokocker, en vanlig sorts bakterie som ger upphov till 30-50 % av samhällsförvärvad lunginflammation i Europa. Bakterien koloniserar luftvägarna hos främst barn, och orsakar även öroninflammationer, bihåleinflammationer, hjärnhinneinflammationer, sepsis (blodförgiftning) och infektioner i leder. Det finns cirka 90 varianter av bakterien, så kallade serotyper, var och en med unika uppsättningar sockermolekyler på sin yta. Ytmolekylerna gör bakterierna mer eller mindre aggressiva i kontakt med människa, och sprids därför olika mycket. Sammanlagt dör cirka 1.5 miljoner människor i världen av pneumokockinfektioner varje år, varav cirka 2/3 är barn eller tillhör andra riskgrupper.

Sedan 1980-talet har det funnits ett så kallat polysackaridvaccin (PPV) som innehåller 23 olika serotyper, det vill säga syntetiska kopior av bakteriernas ytmolekyler. Eftersom immunförsvaret hos små barn svarar dåligt på vaccin som endast innehåller sockermolekyler utvecklades ett nytt sorts vaccin för snart 20 år sedan som kallas konjugerat vaccin (PCV). Sockermolekylerna binds till ett proteinfragment vilket aktiverar immunförsvaret på ett kraftfullare sätt. När resultaten bland små barn blev betydligt bättre med det konjugerade vaccinet började man prova att använda det även till andra riskgrupper som till exempel patienter med reumatiska sjukdomar.

I avhandlingens första delarbete vaccinerades 497 patienter med RA och SpA som följdes vid reumatologkliniken i Lund och Malmö, med en dos 7-valent konjugerat pneumokockvaccin. Antalet pneumokockorsakade infektioner registrerade i Region Skånes Vårddatabas 4 år före respektive efter vaccinationen jämfördes med artritpatienter från Skåne som inte fått vaccinet. Studien visade att de patienter som fått vaccinationen hade en relativt lägre risk för att drabbas av en pneumokockorsakad infektion jämfört med de ovaccinerade artritpatienterna.

I det andra delarbetet användes blodprover som tagits före och en månad efter de ovan nämnda vaccinationerna. Antikroppsnivåer mot 2 sorter av de sockermolekyler (serotyper) som fanns med i vaccinet analyserades. Dessa antikroppsnivåer jämfördes med förekomst av infektion efter vaccinationen. Vi kunde visa att de som drabbades av allvarliga infektioner efter vaccinationen hade bildat signifikant lägre antikroppsnivåer än de som inte fått infektion. Vi beräknade vilka antikroppsnivåer som skulle kunna användas som gränsvärden för att veta om man uppnått tillräckligt skydd eller inte efter en vaccination.

I det tredje delarbetet vaccinerades 47 patienter med SLE och 21 friska individer med en dos 13-valent konjugerat pneumokockvaccin. Antikroppsnivåer analyserades med 3 laborativa metoder och jämfördes mellan olika behandlingsgrupper. Vi fokuserade på ett relativt nytt preparat som heter belimumab, och som ges till SLE patienter med hög sjukdomsaktivitet trots pågående standardbehandling. Belimumab minskar överlevnaden av B-celler, en av de centrala immunceller som är central i sjukdomsuppkomsten av SLE, och som också producerar antikroppar i samband med vaccination eller infektion. De patienter som behandlades med belimumab i tillägg till standardbehandling uppvisade dock inte ett försämrat antikroppssvar jämfört med övriga SLE patienter.

I det fjärde delarbetet ville vi undersöka mekanismer som kan förklara varför det antireumatiska läkemedlet metotrexat hämmar antikroppssvaret efter vaccination. Vår hypotes var att metotrexat minskar antalet antikroppsproducerande celler, så kallade plasmaceller. Tio RA patienter med nydebuterad sjukdom och utan behandling samt 10 RA patienter med pågående metotrexatbehandling vaccinerades med 13-valent konjugatvaccin. De metotrexatbehandlade patienterna hade lägre totalnivåer av antikroppar, samt lägre nivåer och funktion av specifika antikroppar, men däremot sågs ingen skillnad i antalet antikroppsproducerande celler.

Sammantaget visade våra studier att konjugerat pneumokockvaccin, både 7- och 13-valent, kan aktivera immunförsvaret på ett tillfredställande sätt hos patienter med både RA, SpA och SLE även under flertalet pågående antireumatiska behandlingar. Dock noterades liksom vid tidigare studier att antikroppssvaret är sämre hos patienterna än hos friska kontrollpersoner och än lägre hos metotrexatbehandlade patienter. Även om mätning av antikroppar efter vaccination är ett så kallat surrogatmått så korrelerar uppmätta antikroppsnivåer med efterföljande risk att få allvarliga pneumokockinfektioner hos artritpatienter. Belimumab, som teoretiskt

skulle kunna tänkas hämma antikroppssvaret, verkade inte göra det hos våra SLE patienter.

Enligt de aktuella rekommendationerna från både USA och Europa ska alla patienter med IRS vaccineras med både PPV och PCV, men täckningsgraden vid upprepade undersökningar har visat sig vara låg. Inom en och samma diagnosgrupp kan risken att få infektion variera mycket med stora åldersspann och utbredningsgrad av sjukdomen samt ett helt spektrum av antireumatiska behandlingar och doser. Några av våra resultat pekar på behovet av att bättre kunna välja ut vilka patienter som skulle ha allra störst nytta av att vaccineras. Att ta fram verktyg som ett riskindex för sådan bedömning är ett av flera tänkbara spår för framtida studier.

Allgemein wissenschaftliche Zusammenfassung

Rheumatoide Arthritis (RA), Spondylarthritis (SpA), und Systemischer Lupus Erythematosus (SLE) sind Beispiele für Erkrankungen die zum Formenkreis der inflammatorischen rheumatischen Erkrankungen (IRE) gehören. Genetische Variationen und Umweltfaktoren wie zum Beispiel Zigarettenrauch und bakterielle Infektionen im Mund- und Darmbereich spielen eine Rolle bei der Wahrscheinlichkeit diese Erkrankungen zu entwickeln. Die genauen auslösenden Faktoren sind jedoch noch immer nicht bekannt. Gemeinsam für diese Erkrankungen sind Abweichungen im Immunsystem, die zu einer Entzündung (Inflammation) in verschiedenen Organsystemen und diese wiederum zu chronischen Beschwerden und eventuellen bleibenden Schäden führen können.

Neben der Erkrankung selbst, haben die Betroffenen oft mit Nebenerscheinungen, möglichen Folgeerkrankungen und einem erhöhten Risiko andere Erkrankungen wie z.B. Tumoren, Herz- und Gefässerkrankungen und erhöhter Infektionsempfindlichkeit, zu kämpfen. Im Takt mit der Entwicklung moderner antirheumatischer Medizin in den letzten Jahrzehnten hat sich der allgemeine Gesundheitszustand bei Patienten mit rheumatischen Erkrankungen verbessert, die bleibenden chronischen Schäden wie z.B. Gelenkdestruktionen sind vermindert und die Lebenszeit verlängert. Gleichzeitig tragen die Behandlungen jedoch zu einem erhöhten Infektionsrisiko, verglichen mit gesunden Individuen, bei. Fallrapporte von Neuerkrankungen und/oder die Verschlechterung einer bereits bestehenden rheumatischen Erkrankungen im Zusammenhang mit Impfungen gegen Infektionskrankheiten sind beschrieben worden und diese haben zu einer abwartenden Haltung gegenüber Impfungen bei Patienten mit rheumatischen Erkrankungen und den behandelnden Ärzten geführt. Größere kontrollierte Studien konnten jedoch kein erhöhtes Risiko für z.B. das Neuerkranken an RA nach einer Impfung nachweisen und den Patienten werden seit längerer Zeit in Schweden und in anderen Ländern verschiedene Impfungen empfohlen.

In dieser Doktorarbeit (Habilitation) haben wir uns auf die Impfung von Pneumokokken konzentriert; ein Bakterium, das in 30-50 % für Lungenentzündungen in Europa verantwortlich ist. Diese Bakterien kolonisieren die Luftwege vor allem kleiner Kinder und können weiter Ohrenentzündungen, Nasennebenhöhlenentzündungen, Hirnhautentzündungen, Blutvergiftungen und Gelenkinfektionen verursachen. Es gibt circa 90 verschiedene Varianten oder sogenannten Serotypen dieser Bakterien; jede von ihnen charakterisiert durch die verschiedene Zusammensetzung von Zuckermolekülen an ihrer Oberfläche. Diese Oberflächenmoleküle aus Zucker machen die Bakterien mehr oder weniger aggressiv und dadurch mehr oder weniger ansteckend. Jedes Jahr sterben circa 1.5 Millionen Menschen auf der ganzen Welt an einer Pneumokokken Infektion. Davon sind circa 2/3 Kinder oder Angehörige anderer Risikogruppen.

Seit den 80iger Jahren gibt es einen synthetisch hergestellten Polysacharid-Impfstoff (PPV), der 23 verschiedene Serotypen enthält. Da das Immunsystem kleiner Kinder schlecht auf eine Impfung reagiert, die nur Zuckermoleküle enthält, entwickelte man man vor circa 20 Jahren einen neuen Impfstoff, einen sogenannten konjugierten Impfstoff (PCV), an dem die Zuckermoleküle an ein Eiweißmolekül gebunden sind. Dadurch wird das Abwehrsystem der Kinder auf eine kräftigere Art und Weise aktiviert. Als sich die Ergebnisse dieser Impfungen bei kleinen Kindern deutlich verbesserten, fing man an diesen Impfstoff auch bei anderen Risikogruppen wie zum Beispiel Patienten mit rheumatischen Erkrankungen anzuwenden.

In der ersten Teilarbeit wurden 505 RA- und SpA-Patienten der rheumatologischen Klinik in Lund/Malmö mit einem 7-valenten Pneumokokken-Impfstoff geimpft (das bedeutet dieser Impfstoff enthielt Kopien von 7 verschiedenen Öberflächenmoleküle aus Zucker). Die Anzahl Pneumokokken- verursachter Infektionen, die im Region Schonen Gesundheitsregister registriert sind, wurden 4 Jahre vor und nach der Impfung ermittelt und mit ungeimpften Arthritis-Patienten aus Schonen verglichen. Wir konnten zeigen, dass die Patienten, die eine Impfung erhalten hatten, eine relativ niedrigeres Risiko hatten an einer Pneumokokkenverursachten Infektion zu erkranken, verglichen mit den ungeimpften Arthritis-Patienten.

In der zweiten Teilarbeit untersuchten wir Blutproben, die vor und einen Monat nach der Pneumokokkenimpfung abgenommen wurden, und analysierten sie auf Antikörperniveaus von 2 Serotypen des Impfstoffes. Diese Antikörperniveaus wurden dann mit dem Vorkommen einer bakteriellen Infektion nach der Impfung verglichen. Wir konnten zeigen, dass die Patienten, die an einer ernsthaften Infektion nach der Impfung erkrankten, signifikant niedrigere Antikörperniveaus hatten als diejenigen, die keine Infektion hatten. Wir berechneten, welche Antikörperniveaus im klinischen Alltag anwendbar sind, um zu ungefär vorhersagen zu können, ob ein Patient ausreichend nach einer Impfung geschützt ist oder nicht.

In der dritten Teilarbeit wurden 47 Patienten mit SLE und 21 gesunde Kontrollpersonen mit einem 13-valenten Konjugationsimpfstoff geimpft. Die Antikörpertiter wurden mit 3 verschiedenen Labormethoden untersucht und zwischen den beiden Gruppen verglichen. Die SLE-Patienten dieser Studie wurden mit einem relativ neuen, antirheumatischen Medikament behandelt, das bei SLE-Patienten mit hoher Krankheitsaktivät trotz laufender Standardbehandlung angewendet wird. Dieses Medikament, Belimumab, mindert das Überleben von sogenannten B-Zellen, eine Art Immunzellen, die eine zentrale Rolle bei der Entstehung von SLE spielen und die ebenfalls für die Antikörperproduktion nach einer Impfung oder Infektion verantwortlich sind. Die Patienten, die zusätzlich zur Standardbehandlung auch Belumimab erhielten, zeigten doch keine verschlechterte Immunantwort verglichen mit den nicht Belumimab behandelten SLE-Patienten.

In der vierten und letzten Teilarbeit wollten wir die Mechanismen darlegen, die erklären könnten, warum Patienten, die mit dem antirheumatischen Medikament Methotrexat behandelt werden, eine gehemmte Antikörperbildung nach einer Pneumokokkenimpfung zeigen. Unsere Arbeitshypothese war, dass Methotrexat die Anzahl der Antikörperproduzierenden Zellen, den sogenannten Plasmazellen, vermindert. 10 neuerkrankte RA Patienten ohne Behandlung und 10 RA Patienten mit Methotrexatbehandlung wurden mit einem 13-valenten Konjugat-Impfstoff geimpft. Wir konnten zeigen, dass Methotrexatbehandelte Patienten sowohl niedrigere Gesamtantikörperniveaus aufweisen als auch niedrigere Niveaus und eine herabgesetzte Funktion (im Sinne von minderer Wirksamkeit) von spezifischen Antikörpern hatten. Wir konnten jedoch keinen Unterschied in der Anzahl Antikörperproduzierender Zellen, den Plasmazellen, nachweisen.

Insgesamt könnten wir in dieser Doktorarbeit zeigen, dass sowohl der 7- als auch der 13-valente konjungierte Pneumokokkenimpfstoff, eine zufriedenstellende Immunantwort bei Patienten mit RA, SpA und SLE hervorrufen kann, auch unter gleichzeitiger Behandlung mehrerer antirheumatischen Medikamente. Doch wie schon in früheren Studien gezeigt, weisen Methotrexatbehandelte Patienten eine schlechtere Immunantwort als gesunde Kontrollpersonen auf. Auch wenn die Antikörpermessung nach der Impfung nur ein sogenanntes Surrogat-Maß ist (das heißt, dass sie nicht gleichzusetzen sind mit der Impfeirkung), korrelieren doch die gemessenen Antikörperniveaus mit dem folgenden Risiko, an einer ernsthaften Pneumokokkenverursachten Infektion bei Patienten mit rheumatischen Leiden zu erkranken. Das Medikament Belimumab, das rein theoretisch die gesamte Immunantwort hemmen könnte, wirkte dies doch in der Praxis bei unseren SLE-Patienten nicht zu tun.

Laut den aktuellen Richtlinien aus Europa und aus den USA sollen alle Patienten mit einer rheumatischen Erkrankung mit beiden erhältlichen Pneumokokken-Impfstoffen geimpft werden. Dies entspricht aber in Untersuchungen nicht der Realität. Innerhalb ein und derselben Patientengruppe kann das Risiko an einer Infektion zu erkranken sehr unterschiedlich sein. Altersunterschiede, unterschiedliche Krankheitsaktivität und die breite Spannweite an antirheumatischen Behandlungen und ihrer unterschiedlichen Dosierungen sind mögliche Erklärungen. Einige Ergebnisse weisen auf die Notwendigkeit hin, besser Vorhersagen zu können, welche Patienten den größten Nutzen einer Impfung gegen Pneumokokken haben könnten. Ein geeignetes Beurteilungs-Instrument zu entwickeln, z.B. ein Risikoindex, wäre eine denkbare Arbeit für zukünftige Forschung in diesem Bereich.

Introduction

Inflammatory rheumatic diseases (IRDs) are conditions in which cells and cytokines of the immune system induce inflammatory reactions, targeting body tissues as if they were non-self, causing organ damage. The primary focus of symptoms and impairment varies; joints in arthritis, muscles in myositis, blood vessels in vasculitis e.t.c, but the diseases can also overlap each other, spread and give systemic effects i.e fatigue and elevated risks of cardiovascular disease, malignancies and infections.[1-5]

Patients with IRD have an increased risk of infection. [5-10] This is not only due to the diseases or their associated comorbidities, but also to the treatment. The conventional synthetic disease modifying antirheumatic drugs (csDMARDs) have been in use for several decades with somewhat elevated risks of infections but without imposing a larger clinical problem. Glucocorticoids have among other side effects a well known and documented association with increased risk of infections, but are still broadly used and have a crucial role in IRD treatment, especially in times of flares and severe disease.[11] During the last decades, several so called biological treatments i.e anti-TNF, anti-IL-6, anti-CD20, anti-IL17 and anti-BlyS have been developed and are now available to patients in most industrialized countries. These drugs have had positive impact on the management of disease and the patients chance of remission, but also imposes new challenges regarding side effects, such as infections.[12]

Streptococcus pneumonia is a common bacterium worldwide, killing about 1.5 million people each year.[13] It causes several kinds of infections; pneumonia, meningitis, sepsis, otitis, and septic arthritis among others. In adults, the primary risk individuals are immunodeficient persons and elderely adults with comorbidities, but also adults in close contact with children. [14-16]

Preventive steps have been taken since the 1980ies with a wide spread use of the 23-valent polysaccharide pneumococcal vaccine (PPV), although showing diverging results regarding efficacy against invasive and non-invasive illness in different settings.[17,18] In the year 2000 a new type of vaccine was launched, initially for children < 2 years of age. A conjugated peptide was connected to the capsular polysaccharide in order to stimulate the immature immune system in a more effective manner. Soon after, this conjugated pneumococcal vaccine (PCV) was tried out on adult risk individuals such as HIV-patients or splenectomized patients.[19] Today, both PPV and PCV are included in international and Swedish

recommendations for adults with a number of immunocomprising conditions, including IRD.[20-23]

The overall purpose of this thesis was to further investigate the factors which influence the protective effect of conjugated pneumococcal vaccination given to IRD patients (rheumatoid arthritis, spondyloarthritis and systemic lupus erythematosus) with different ongoing immunosuppressant treatments measured both as humoral response as well as registered infections.

Rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic inflammatory disease, typically characterized by symmetrical swelling, pain and dysfunction of small joints, the occurrence of autoantibodies and permanent tissue damage of cartilage and bone.[24] The extra-articular manifestations and comorbidities associated include among others rheumatic nodules, interstitial lung disease, cardiovascular disease, elevated risk of certain malignancies and a decreased life expectancy. [25-27] The prevalence in Western Europe and North America is about 0.5-1.1%, with 20-50 new cases per 100,000 inhabitants and year.[28-30] Two thirds of RA patients are women and the incidence increases with age. The median age of disease onset around 55-60 years of age. [31]

Etiology and pathophysiology

The probability of developing RA depends on a combination of genetic predisposition and exposure to environmental factors.[32] Several different immune cells, cytokines and signalling pathways are involved in the pathophysiology of RA, creating a vicious circle. However, the starting point remains unknown.

The inner surface of a joint consists of fibroblast-like synoviocytes producing the synovial fluid. When inflamed, the synovium is infiltrated by several different cell populations of the immune system e.g. macrophages, B- and T cells. The normal protective mechanisms of both synovium and cartilage are disturbed, chondrocytes undergo apoptosis, and potent cytokines promote differentiation and invasion of osteoclasts in the periosteal surface leading to bone erosion. [24,33]The cytokines involved in promoting the tissue damage are numerous, and the pattern differs with time and stage of the disease. Among others, IL-6 and TNF both have a central role in the inflammatory cascade. IL-6 activates leucocytes and stimulates antibody production, but also mediates more systemic effects such as anemia and lipid metabolism dysregulation. TNF stimulates the cascade of numerous other cytokines and chemokines, resulting in extravasation of inflammatory cells, angiogenesis and induction of pain.[34,35]

50-80% of RA patients produce one or both of the typical autoantibodies called rheumatoid factors (RF) and anti citrullinated-peptide antibodies (ACPA), sometimes occurring in the circulation several years before the clinical onset of

symptoms.[36] RF are antibodies directed towards the Fc region of immunoglobulin G, and can be found among patients with a variety of autoimmune diseases, as well as among 4 % of the healthy Caucasian population.[37]

ACPA are, as the name implies, directed towards the amino acid citrulline. The transformation of arginine to citrulline is thought to be enhanced by several potential exposures such as smoking and infections. [38,39] The loss of tolerance leading to production of these autoantibodies is also associated with variants of the major histocompability complex HLA-DRB1, called the ”shared epitope”.[40,41]

Cigarette smoking is the best established environmental risk factor for RA, not only increasing the risk of the disease, but also associated with more severe and early joint damage, and poor response to treatment.[42-44]

Clinical features

RA most often involve the smaller joints of the hands and feet, where inflammation results in joint swelling, pain, stiffness and reduced strength and mobility. It can spread to all joints of the body, potentially causing tissue damage and deformities when left untreated. [45] In recent years, the importance of early diagnosis and initiation of effective treatment in order to reach sustainable remission has become more evident.[46,47] In order to find RA patients earlier than before, the ACR classification criteria from 1987[48] were replaced in 2010.[49]; see *Table 1*. There are several different scoring systems to evaluate disease activity and disability of arthritis patients. The patient’s symptoms, joint counts, objective measures of inflammation as CRP or ESR, and assessment of how the disease affects activities of daily living are weighed together. The Disease activity score 28 (DAS28) and the Health Assessment Questionnaire (HAQ) were used in our studies, and are included in the tables of demographic and disease characteristics of the cohorts. DAS28 comprises 28 swollen and tender joints (0-28), ESR (mm/h) or CRP (DAS28CRP) and a general health assessment (VAS, visual analogue scale). [50] The HAQ consists of 20 questions in 8 different domains assessing the level of disability with regard to daily activities as eating, dressing, walking and gripping etc.[51,52]

Extra-articular disease, comorbidities and mortality

Extra-articular manifestations of RA (ExRA) can occur in most parts of the body and internal organs, e.g. rheumatic noduli in subcutaneous tissue, keratoconjunctivitis sicca of the eyes, vasculitis of the skin or around nerves, pleuritis, pericarditis, glomerulonephritis or interstitial lung disease (ILD). ILD comprises a heterogenous group of parenchymal lung disorders, giving different radiological features. The prevalence of ILD among RA patients vary substantially between different studies (10-67 %). The symptoms include fatigue, cough, dyspnea and increased risk of respiratory tract infection.[53] Prognostic factors for developing ExRA manifestations are several; smoking, poor disease control and high disease activity, as well as seropositivity (ACPA or RF positive disease)[54,55] RA in general and active disease in particular also elevates the risk of several comorbidities, such as cardiovascular disease, certain malignancies, depression, chronic obstructive lung disease as well as severe infections. Even if the proportion of these patients have declined during the last decades, probably due to more efficient treatment and closer monitoring, both morbidity and mortality of patients with RA are still increased in comparison to the general population.[25,56,57]

Infections in RA

Patients with RA have about twice the risk of acquiring serious infections than the healthy population, and equally a higher risk of being hospitalized and dying from the infection.[7,8 ,58] The most common sites of infections are the respiratory tract, the genitourinary tract, and the joints. The elevated risk of being infected is associated with the severity and activity of RA. Furthermore, increasing age, comorbidities such as cardiovascular disease, renal failure, interstitial lung disease and previous history of serious infections adds on to the risk. [4,6,9] Glucocorticoids and several modern antirheumatic drugs such as anti-TNF and anti-CD20 (rituximab) have been shown to further increase this risk. [4,11,12,59,60]In case of infection, DMARD treatment is often discontinued, leading to a vicious circle of even higher disease activity, need of more glucocorticoids and thus an elevated risk of repeated infections. Several different risk scoring systems for RA patients have been developed in order to identify patients with higher risk of infection, but with limited implementation in the clinic.[6,61,62]

Table 1.
ACR Classification criteria for RA from 2010 and 1987. Patients scoring ≥6 or fulfilling ≥4 criteria respectively are classified as having RA.

The 2010 ACR/EULAR classification criteria for RA		**The 1987 ACR classification criteria for RA**	
Target population: 1) Patients with at least 1 joint with definite clinical synovitis 2) Synovitis not better explained by another disease	Score	1. **MORNING STIFFNESS**	Morning stiffness in and around the joints, lasting at least 1 h before maximal improvement.
A) JOINT DISTRIBUTION		**2.ARTHRITIS IN THREE OR MORE JOINT AREAS***	Soft tissue swelling or fluid present simultaneously for at least six weeks.
1 large joint*	0		
2-10 large joints	1	**3. ARTHRITIS OF HAND JOINTS**	Swelling of wrist, MCP or PIP joints for at least six weeks
1-3 small joints** (irrespective of large joint involvement)	2		
4-10 joints (irrespective of large joint involvement)	3	**4. SYMMETRICAL ARTHRITIS**	Simultaneous involvement of the same joint areas (as defined in 2) on both sides of the body (bilateral involvement of PIPS, MCPs, or MTPs is acceptable without absolute symmetry) for at least six weeks
>10 joints (with ≥ 1 small joint)	5		
B) SEROLOGY		**5. RHEUMATOID NODULES**	Subcutaneous nodules, over bony prominences, or extensor surfaces, or in juxta articular regions
Negative RF and ACPA	0		
Low positive RF or ACPA	2	**6. SERUM RHEUMATOID FACTOR**	Demonstration of abnormal amounts of serum rheumatoid factor by any method for which the result has been positive in 4 % of normal control subjects
High positive RF or ACPA	3		
C) ACUTE-PHASE REACTANT		**7. RADIOGRAPHIC CHANGES**	Typical RA changes on hand and wrist radiographs, which must include erosions or unequivocal bony decalcification localized in or most marked adjacent to the involved joints (osteoarthritis changes alone do not qualify)
Normal CRP and ESR	0		
Abnormal CRP or ESR	1		
D) DURATION OF SYMPTOMS			
< 6 weeks	0		
≥ 6 weeks	1		
*Shoulders, elbows, hips, knees, ankles ** PIP-joints, MCP-joints, MTP-joints, wrists		*Possible areas: right or left PIP-, MCP-, wrist, elbow, MTP-, knee or ankle joints	

Spondyloarthritis

The umbrella term spondyloarthritis (SpA) includes several rheumatic arthritic diseases, all with their own characteristics as well as a large portion of overlapping features including symptoms, organ involvement, etiology and treatment, affecting about 0.5-1.5% of the population in Western countries. [63]Inflammatory back pain disease or axial SpA includes ankylosing spondylitis (AS) and non-radiographic SpA (nr-SpA) with the main symptomatic focus in the lower back and sacroiliac joints. Psoriatic arthritis (PsA) associated with the skin disease psoriasis, enteropathic spondyloarthritis (EA) associated with inflammatory bowel disease (IBD), and reactive arthritis (ReA) are often peripheral forms of SpA with classic joint involvement as well as daktylitis, enthesitis and tenosynovitis, but might as well include symptoms from the back. Undifferentiated SpA is a heterogenous group with a mixture of inflammatory symptoms from both the back and peripheral joints and other organs, but without a typical picture of a certain form of SpA. The prevalence of different forms of SpA varies greatly between populations around the world and covaries with the percentage of HLA-B27. Results from southern Sweden by Haglund et al showed an overall prevalence of SpA of 0.45% with PsA and AS being the largest subtypes. [64] PsA affects up to 30% of patients with psoriatic skin disease.[65] About 10-20% of patients with IBD such as Crohn's disease and Ulcerative colitis suffer from peripheral forms of SpA and about 5% of them develop an axial form of SpA.[66] Reactive arthritis is typically an asymmetrical oligoarthritis, dactylitis or enthesitis occurring within 6 weeks after a gastrointestinal or urogenital infection, and wears off within a few months.

Pathogenesis

The subtype HLA B27 of MHC class I antigens is one of the most abundant versions in white humans. The genetic correlation to SpA was discovered already in the 70ies and is still the strongest one known today.[67,68] The prevalence of HLA B27 is higher in northern Scandinavia (10-15%) and Canada and lower in sub-Saharan Africa, and the occurrence of SpA diseases covaries roughly accordingly.[69] Different hypotheses of how the molecule contributes to the inflammatory response include e.g. molecular mimicry and cross reactivity between pathogens and self-peptides as well as misfolding of the molecule itself triggering an intracellular immune response. [70] More than 70% of AS patients in Sweden are HLA B27 positive,

whereas the percentages in other forms of SpA are much lower. [66]The composition of the human gut microbiome is of large interest in ongoing research of the pathogenesis of SpA. Several microorganisms have been identified, but further studies are needed to fully understand the pathogenic mechanisms.[71]

Clinical features

Apart from symptoms from the locomotor system described above, SpA patients often suffer from a number of extra-articular manifestations, e.g. psoriasis in the skin, anterior uveitis of the eye and inflammatory bowel disease.[72]In 2011, the Assessment of SpondyloArthritis international Society (ASAS) published a new set of classification criteria for "peripheral SpA and SpA in general", giving the different manifestations slightly differing significance in the classification scheme, see *figure* .[73] The disease activity and symptoms caused by inflammation are assessed with a couple of different indices such as Bath ankylosing spondylitis disease activity index (BASDAI) which consists of the patient's self-reported symptoms such as fatigue, morning stiffness and pain from peripheral or axial joints. The ankylosing spondylitis disease activity score (ASDAS) also consists of objective features such as acute phase reactants.[74,75] Health assessment questionnaire (HAQ), described in the RA chapter is used also in the assessment of SpA disease. Comorbidities associated to SpA include cardiovascular disease with both ischemic heart disease and cerebrovascular disease, and increased risk of certain malignancies. [76-78]

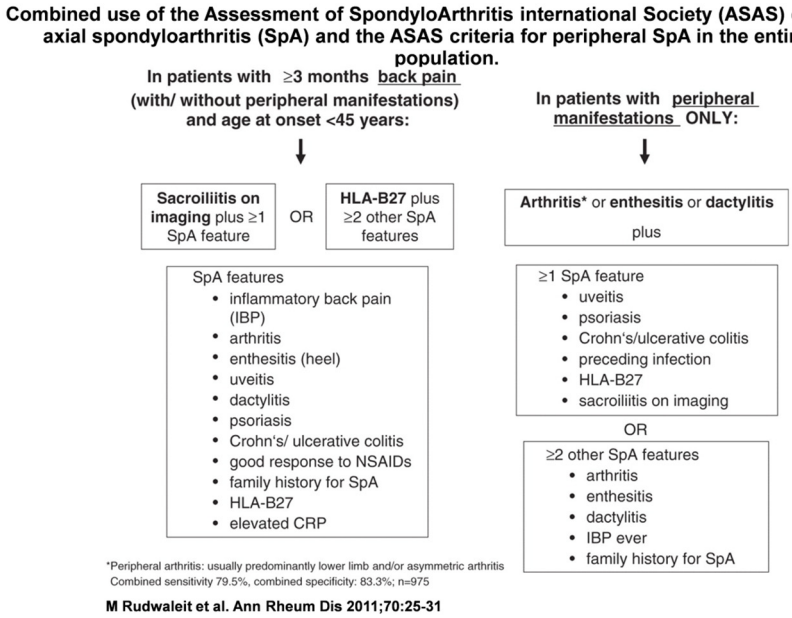

Figure 1.
Assessment of SpondyloArthritis international Society (ASAS) criteria for axial and peripheral spondyloarthritis (SpA).[73]

Infections in spondyloarthritis

The infection rates among patients with SpA have repeatedly been reported to be elevated compared to healthy subjects, however seemingly somewhat lower than among RA patients.[79,80] In one large retrospective cohort study from the UK including 20 000 AS patients, the risk of hospitalization for pneumococcal infection was significantly increased (rate ratio 2.53, 95% CI 2.32-2.75)[8] compared to healthy individuals. However, another observational study of 440 patients with axial SpA and different kinds of treatment showed surprisingly low numbers of infections.[81] The types of infections and major pathogenic microorganisms seem to be the same as in other rheumatic disorders. More than 20% of the deaths among AS patients in a Norwegian cohort were caused by infections, compared to 6 % in the general population. [82]

Systemic lupus erythematosus (SLE)

Systemic lupus erythematosus (SLE) is an autoimmune disease, targeting multiple organs. The severity of disease ranges between very mild skin or joint involvement to multiple and chronic organ damage and comorbidities as well as increased mortality. The treatment differs accordingly between only NSAIDs or antimalarial substances, to heavy immunosuppressants. 90 % of the patients are women, with a peak incidence in the fertile years between 15-40 years of age.[83] In Sweden, the prevalence is about 60 /100 000 inhabitants [84] in comparison to estimates of prevalences in different parts of the world ranging between 7-160 /100 000. [85]

Etiology and pathophysiology

The risk of developing SLE is associated with a number of genetic variants leading to disturbances of the immune system. Several environmental risk factors such as ultraviolet light, smoking, drugs and possible infectious triggers have also been identified. [1] Disturbances in both the innate and the adaptive immune system contribute to a loss of self-tolerance, and the production of large amounts of nuclear autoantibodies such as anti-dsDNA, anti-C1q, anti-Ro, anti-Sm. The interplay between B- T- and dendritic cells does somehow become overactive, leading to increased levels of cytokines such as IFNα , IL-6 and TNF which further stimulate the B cells. Autoantibodies and antigen from apoptotic cells form immune complex who are deposited in e.g. the blood vessels, causing vasculitis. Defect phagocytosis and hypocomplementemia contribute to an impaired clearance of apoptotic material from the circulation, further triggering the inflammatory cascade. Among other cytokine abnormalities, high levels of B lymphocyte stimulating factor (BlyS) are seen, inhibiting apoptosis of the autoreactive B cells. [86,87]

Biomarkers for the diagnosis of SLE include antinuclear antibodies (ANA) which is a hallmark of SLE and is included in the ACR classification criteria [88,89] (*Table 2*) The different extractable nuclear antigens (ENA) giving the positive immunofluorescent patterns of ANA can be several; anti-Ro/SSA, anti-La/SSB, anti-Sm among others. Occurrence of anti-dsDNA and decreased levels of complement proteins (hypocomplementemia) are also both characteristic findings of SLE and can be seen to fluctuate with disease activity. Cytopenias are seen in all

cell lines of the bone marrow; trombocytopenia, leukopenia and inflammatory or hemolytic anemia.[90,91]

Clinical features

SLE includes a broad and heterogenous range of phenotypes with diverging symptoms, immunological patterns, subgroups and time perspectives. Development of diagnostic criteria and indices of disease activity and organ damage as prognostic factors and preclinical findings contribute to map out the disease in each patient [88,89] (*Table 2*) Typical symptoms include a reddish skin erythema of the face, called malar rash, possibly looking like a bite of a wolf which has given name to the disease, as *lupus* is Latin for wolf. Other manifestations include arthritis of the joints, serositis of the lungs and heart (pleuritis, myocarditis), myositis, nephropathy and vasculitis of the brain with risk of severe and persistent organ damage. [92] *Cervera et al* presented a comparison of the abundance of the clinical manifestations between their cohort and three other prospective studies from different parts of the world with significantly differing findings of malar rash (31-76%), photosensitivity (23-60%), arthritis (48-88%), nephropathy (28-74%), and neurologic involvement (19-23%).[93] This illustrates the heterogenicity of the clinical picture which also seems to alter depending on age at onset, gender, disease duration as well as occurrence and level of anti-dsDNA among other factors. To map out the phenotype in each individual patient, different indices including current disease activity, disease history and persistent organ damage have been developed. The most commonly index to assess disease activity is the Systemic Lupus Erythematosus Disease Activity Index or SLEDAI, with the current version from year 2000 called SLEDAI-2K. It includes both symptoms from different organ systems as rash, arthralgia, mental disturbances, seizures or muscle weakness, as well as laboratory findings as proteinuria, hypocomplementemia or cytopenia. [94]

Table 2.
The 1982 revised criteria for classification of systemic lupus erythematosus with addition of the added criteria from 1997.

The 1982 revised criteria for classification of systemic lupus erythematosus	
Criterion	**Definition**
1.Malar rash	Fixed erythema, flat or raised, over the malar eminences, tending to spare the nasolabial folds
2.Discoid rash	Erythematous raised patches with adherent keratotic scaling and follicular plugging; atrophic scarring may occur in older lesions
3.Photosensitivity	Skin rash as a result of unusual reaction to sunlight, by patient history or physician observation
4.Oral ulcers	Oral or nasopharyngeal ulceration, usually painless, observed by a physician
5.Arthritis	Nonerosive arthritis involving 2 or more peripheral joints, characterized by tenderness, swelling, or effusion
6.Serositis	a) Pleuritis-convincing history of pleuritic pain or evidence of pleural effusion OR b) Pericarditis documented by ECG or evidence of pericardial effusion
7.Renal disorder	a) Persistent proteinuria greater than 0.5 grams per day or greater than 3+ if quantitation not peformed OR b) Cellular casts-may be red cell, hemoglobin, granular, tubular, or mixed
8.Neurologic disorder	a) Seizures-in the absence of offending drugs or known metabolic derangements; e.g., uremia, ketoacidosis, or electrolyte imbalance OR b) Psychosis in the absence of offending drugs or known metabolic derangements, e.g., uremia, ketoac- idosis, or electrolyte imbalance
9.Hematologic disorder	a) Hemolytic anemia-with reticulocytosis , b) Leukopenia, c) Lymphopenia, d) Thrombocytopenia
10.Immunologic disorder	a). Positive LE cell preparation , b) Anti-DNA: antibody to native DNA in abnormal titer , c) Positive Anti-Sm, d) False positive serologic test for syphilis Revision 1997: Positive aCL or LA instead of positive LE cell preparation
11.Antinuclear antibody	An abnormal titer of antinuclear antibody by immunofluorescence or an equivalent assay at any point in time and in the absence of drugs known to be associ- ated with “drug-induced lupus” syndrome
A person shall be said to have systemic lupus erythematosus if any 4 or more of the 11 criteria are present, serially or simultaneously, during any interval of observation.	

Comorbidities and mortality in SLE

SLE patients have elevated risks of multiple comorbidities. In recent decades, the survival of SLE patients have improved, but all-cause mortality is still significantly higher than the general population, with major causes of death being cardiovascular disease (CVD), malignancies, infections and complications to renal failure. [95] The risk of CVD is increased 2.7-5-fold compared to the general population, possibly due to traditional risk factors as hypertension and smoking, but also associated with inflammatory activity and use of glucocorticoids.[96-98] Anti phospholipid antibody syndrome (APS) is a thromboembolic disorder including autoantibodies towards proteins in cell membranes, (lupus anticoagulant, anticardiolipin IgG, anti-β2-glycoprotein) so called aPL. About 30-50% of SLE patients have one or more of the known aPL, and about 15-20% develop APS with clinical manifestations of both arterial and venous thrombotic events, affecting both morbidity and mortality. [99]A metaanalysis from 2015 affirmed an elevated risk of overall cancer, with the most

abundant types being non-Hodgkin lymphoma, Hodgkin lymphoma and leukemia. [100]

Infections in SLE

Infections are a major cause of both morbidity and mortality in SLE patients. About half of the patients will be hospitalized because of an infection during the course of their disease, and half of the deadly infections are pneumonias. [10,101] Common bacteria such as Streptococcus pneumoniae, Echerichia coli and Staphylococcus aureus are the most prevalent, but also opportunistic infections e.g. Varicella Zoster, Candida, Pneumocystis jirovecii, and cytomegalovirus (CMV) are abundant. [5]The complex immunological disturbances including both the innate and humoral response in SLE have been identified as the largest explanatory factor for these elevated risks, but also damages to the epithelial barriers of skin rashes and ulcers. Elevated disease activity (SLEDAI > 8), kidney involvement, hypocomplementemia and neutropenia have been identified as risk factors of invasive infections, as well as some of the treatment regimens. [102]

Antirheumatic treatment

Antirheumatic treatment is chosen based on diagnosis of disease, current level of inflammation, organ damage, prognostic factors and presence of contraindications relevant to the individual patient. The specific antirheumatic drugs, often called disease modifying antirheumatic drugs or DMARDs, can be divided into two main groups according to the method of manufacture: synthetic (sDMARDs) or biologic (bDMARDs). Within the group of sDMARDs a subgroup of targeted drugs, the JAK-inhibitors, have recently been introduced. Other groups of drugs often used are nonsteroidal anti-inflammatory drugs and glucocorticoids.

The drugs used in Paper I-IV are briefly described below.

Nonsteroidal anti-inflammatory drugs (NSAIDs, COX-inhibitors) can be used successfully in early and mild SpA, but are seldom sufficient for treatment of IRD, if not combined with more potent drugs. They inhibit prostaglandins, which play a role both in inflammation, pain conduction and fever, by blocking the cyclooxygenase enzymes (COX-1 and COX-2).[103] No studies to our knowledge have shown an increased risk of infection or a decreased antibody response to vaccinations among IRD patients with ongoing NSAID treatment.

Glucocorticoids (GC) are a group of drugs originating from the endogenous hormones of the adrenal gland. The fast and potent anti-inflammatory effect was first demonstrated among RA patients, and the discovery was rewarded the Nobel Prize in 1950. Synthetic versions of GC are still a crucial part of the antirheumatic armamentarium, especially during flares and severe organ involvement of IRD. GC bind to their receptors intracellularly. The complex enters the nucleus, binds to DNA and inhibits the expression and production of several cytokines, i.e. IL-2, IL-6, COX-2 and TNF. [104] GC treatment have repeatedly been shown to increase the risk of different kinds of infections, especially among patients on sustained high dose treatment of ≥ 20 mg prednisolone /day.[105] Accordingly, the humoral response has been impaired among GC treated IRD patients in studies of vaccination efficacy, however to a varying extent. [106]

Methotrexate (MTX) was originally developed for hematological malignancies, but has a strong antirheumatic effect in low dosages (≤ 25 mg/week). It is a folic acid antagonist but the mode of action in arthritis is not entirely clear. It inhibits a key enzyme; dihydrofolate reductase, required in the *de novo* synthesis of DNA and RNA, mostly affecting cell populations with high turnover such as inflammatory cells. [107,108] The decrease in dihydrofolate reductase also leads to lower production

of lymphotoxic products via reduced polyamines. One MTX metabolite, the MTX polyglutamate, can through other enzymatic steps induce increased levels and accumulation of adenosine. This adenosine pathway has been shown to induce a number of anti-inflammatory effects, as well as being involved in some of the side effects of MTX. One of the impacts of adenosine is decreases in levels of TNF and IL-6, and elevated levels of IL-10.[107-109] In higher dosages, MTX is clearly immunosuppressant with increased risks of cytopenias, but the studies of patients with antirheumatic doses have shown both significantly elevated risks of infection as well as no risk increase. [9] Antibody response among IRD patients on MTX is however clearly impaired according to a number of studies, both quantitatively and qualitatively, after different immunizations including influenza, PPV and PCV. [110-112] The exact mechanisms behind this impairment has however not been elucidated despite efforts to analyse different maturational stages of lymphocytes. [34,113]

Sulfasalazine (SSZ) is a combination of the antibiotic sulfa and acetylsalicylic acid and was originally invented for treatment of RA by professor Nanna Svartz in the 1940-ies. It is used for treatment of arthritis patients in combination with MTX and hydroxychloroquine or when MTX is not tolerated. It can cause bone marrow suppression with leukopenia and hemolytic anemia, potentially leading to increased risk of infection.[114]

Azathioprine (AZA) is another of the older immunosuppressive drugs used in several medical fields. In SLE, AZA has its main indication in sustaining remission after nephritis, but also in order to taper glucocorticoid doses in skin and joint involvement. [115] The metabolites of the drug are incorporated in RNA and DNA, causing inhibition of proliferation of T and B cells.[116-118] The risk of pancytopenia increases the risk of infection, but to a lower degree than for example glucocorticoid treatment. AZA has been shown to somewhat reduce antibody response following pneumococcal or influenza vaccination among SLE patients. [119]

Hydroxychloroquine (HCQ) is a synthetic version of the antimalarial drug chloroquine, with well-established antirheumatic effects and an anchor drug in SLE treatment. It affects the intracellular pH resulting in decreased secretion of proteases and cytokines, and impairs the lymphocyte proliferation, but the mechanisms responsible for the antirheumatic effects are not fully elucidated. [120-122] The side effects are considered mild, and both in vitro and in vivo studies have shown protective effects against bacterial, fungal and viral pathogens, and against serious infections among lupus patients.[5,10]

Anti-TNF treatment (infliximab, adalimumab, etanercept, golimumab and certolizumab) was the first bDMARD introduced and has been available in the clinic since the late 1990-ies. They inhibit the cytokine TNF, a key element in the inflammatory cascade. In arthritic disease, the therapy leads to significant decreases in pain, stiffness and swollen joints as well as reduced joint damage, and is preferably combined with MTX or other csDMARD. [123] Systematic reviews and meta-analyses have reached different conclusions as to whether anti-TNF treatment

is a significant risk factor of infection or not. [79,124,125] In the most recent meta-analysis in The Lancet from 2015, *Singh et al* includes 106 randomized trials, finding a significant risk increase of serious infections, both on standard dose (OR 1.31, 95% CI 1.09-1.58) and high-dose treatment with bDMARD (OR1.90, 95% CI 1.50-2.39), but not on low dose. [12] The antibody response however, does not seem to be impaired after vaccinations against influenza or pneumococci among anti-TNF treated arthritis patients [110,112]

Anti-BlyS; Belimumab is a recent addition to the bDMARDs. It can be given to SLE patients with moderate to high disease activity despite ongoing standard of care treatment. It is a monoclonal antibody, targeting one survival factor of the B cells; soluble B lymphocyte stimulator, also called BlyS. When BlyS is hampered, a larger proportion of the B cell turn apoptotic, and the levels of autoantibodies decrease. [126,127]However, reports of adverse events after seven years of treatment showed no significant increase in risk of infection. [5,128]

Streptococcus pneumoniae

Streptococcus pneumoniae, also called pneumococcus, was discovered in the late 19th century by Pasteur and Sternberg. [129]It is a major human pathogen, and the leading cause of pneumonia both among children and adults around the world, killing about 1.5 million people each year [13] Pneumococcal disease (PD) is usually divided into invasive and non-invasive, where the latter consists of sinusitis, acute otitis media and community-acquired pneumonia (CAP). In 20-60% of the cases of CAP, no pathogen is found, but it has been estimated that one to two thirds of bacterial pneumonia is caused by pneumococci. [14] Invasive pneumococcal disease (IPD) is defined as an infection with positive findings of *S pneumoniae* in normally sterile fluids as e.g. blood, cerebrospinal or synovial fluid in cases of bacteraemia, meningitis or bacterial arthritis.

The bacteria have its natural reservoir in the nasopharynx of humans, mainly in children. The virulence of the bacteria is mainly due to its polysaccharide capsule with > 90 different identified serotypes, each with a unique set of polysaccharide molecules covering the surface of the bacterial capsule. [129] The capsule enables the bacteria to evade phagocytosis. [130]Only 20-30 of the serotypes have shown significant invasiveness, that is the ratio between the rate of invasive pneumococcal disease (IPD) of one serotype, to the rate of asymptomatic colonisation for the same serotype.[131] Some serotypes are more prone to cause infection in children, others among older adults. The capacity to elicit an antibody response or to activate the complement cascade also differ between the serotypes, making the less immunogenic serotypes more virulent and associated with longer colonisation and more severe disease.[129,132] The serotypes also differs in their capacity in taking up extracellular DNA from the environment, and thus their ability to develop antibiotic resistance.[133] The geographical distribution of the serotypes also differs between countries and continents, and is continuously changing in relation to the usage of pneumococcal vaccines.

The total costs related to CAP in Europe have been estimated to be about €10 billion annually, including both direct and indirect costs. The estimated cost per episode of pneumococcal pneumonia was about €2500 in Europe, while in the USA the cost per episode of CAP was between $7000-8000.[14] In comparison, the cost for one acute myocardial infarction is about €4000-5000 in Europe.[134]

Pneumococcal vaccines

The development of pneumococcal vaccines has been in progress for more than one hundred years. It started with whole cell vaccines, tried out among miners in South Africa in the early 1910s. [129]Thanks to more advanced typing systems, the serotype specific polysaccharide vaccines (PPV) were introduced in the 1940s with valencies from 2-23 serotypes included. In the year 2000 the protein-conjugated polysaccharide vaccines (PCV) were launched in order to reach infants and young children, including 7-15 serotypes. Both PPV and PCV are now internationally recommended to risk individuals and elderly worldwide. [20,21]

Historically, there have been a number of case reports on induction of rheumatic diseases in healthy subjects after different kinds of vaccination, e.g. arthritis development after hepatitis B or tetanus immunization. [135] [136] However, the causality has never been proven in larger case-control studies. [137] Instead, natural infections of e.g. rubella have been shown to increase the risk of developing arthritis. Case reports concerning the risk of vaccinations triggering flares and impairment of symptoms among patients with a known rheumatic disease, e.g. influenza vaccination among SLE patients, have also been published. [138] Correspondingly, larger controlled trials with RA and SLE patients with low disease activity or stable remission has not been able to show any augmented risk of flares. Among patients with an active ongoing flare, the risk of impairment is unknown, and accordingly vaccines should preferably be given during a stable phase of disease. [23]

Partly because of the uncertainties mentioned above, the coverage of pneumococcal vaccination among patients with IRD has been and is still suboptimal according to repeated estimates. In some countries it is part of the routine to always assess the vaccination status prior to starting treatment, but the coverage differs greatly between clinics, regions and countries. *Hmamouchi et al* reported in 2015 from the COMORA cohort including almost 4000 RA patients in 17 different countries an aggregated pneumococcal vaccine coverage of 17 %, with huge disparity between 0 % in Marocco and 57% in France. In multivariate analysis, the predictive factors among RA patients of getting a pneumococcal vaccination were: age > 65 years, biological DMARD treatment, absence of glucocorticoids, and high level of education. [139] Pneumococcal and influenza vaccine uptake among SLE patients in a survey showed that about 40% had received both types, however only patients with health insurance were included. [140] The knowledge and attitude of the treating rheumatology specialist, as well as concerns of the patients are strongly associated to the level of uptake.[141,142]

Polysaccharide pneumococcal vaccine (PPV)

The currently available 23-valent polysaccharide vaccine, in use since the 1980s, is given to adults > 65 years and younger adults with any risk conditions for invasive pneumococcal disease. Diverging results have been published regarding the clinical efficacy of PPV23. Some studies are showing results of protection against IPD among the general adult population, but no certain benefit have been shown when it comes to community acquired pneumonia (CAP), other non-invasive disease, or invasive disease among risk individuals having immunocomprising conditions, elderly adults or HIV-infected patients.[18,130]

Polysaccharide antigens cannot be presented on the major histocompatibility complex (MHC) class II of antigen presenting cells (APC), nor stimulate $CD4^+$ T helper cells. Instead mainly subpopulations of B cells abundant in the marginal zone of the spleen and the mucosa are activated via B cell receptors with some involvement of complement factors. These B cells differentiate into short-lived plasma cells producing mainly IgM antibodies. Isotype switching to IgG and IgA, development of immunological memory and high affinity antibodies are limited. [143]

The largest burden of pneumococcal disease strikes small children who have a particularly poor T cell independent immune response. This was the reason for developing the protein conjugated pneumococcal vaccines (PCV), see below. [144] PPV23 is however still included in the recommendations to be given to risk individuals such as patients with IRD in combination with PCV. Reports of hyporesponsiveness following repeated doses of PPV23, as well as low opsonic capacity of the antibodies is thought to be associated with higher age.[130]

Conjugated pneumococcal vaccine (PCV)

The fact that a stronger antibody response could be obtained by conjugating the bacterial polysaccharide to a carrier protein was discovered already in the 1920ies. [145] The first conjugated pneumococcal vaccine was however licensed in the year 2000 in the United States and introduced in the Swedish immunization program for children in 2009. [146,147] Each of the seven capsular polysaccharides are conjugated to the non-toxic mutant of diphtheria toxin (CRM197). The carrier was chosen in order to additionally function as a booster if the recipient already had been immunized against diphtheria. [145]After the first 7-valent (including 7 serotypes) vaccine was introduced, the 10- 13- and 15-valent versions have been developed.

The vaccine polysaccharides and their conjugated protein can be presented on MHC class II molecules of APCs, and will thereby activate follicular B cells with the help of $CD4^+$ T cells. This process takes place in germinal centres of the lymph nodes where-after some of the B cells differentiate into long-lived plasma cells and migrate

to the bone marrow. The antibody production includes isotype switching to IgG and IgA and production of high affinity immunoglobulins in large amounts. Memory B cells in the bone marrow partly continue to produce antibodies for many years, whereas some stay passive until stimulated by a subsequent infection.[143,148]

PCV induces a mucosal immunity leading to significant decrease in bacterial colonisation of the nasopharynx in vaccinated children. This leads not only to decrease of IPD and hospitalizations among the children but also protection among unvaccinated adults in the same community, so called herd immunity.[130] Among healthy adults > 65 years of age, PCV13 was shown to reduce the risk with 45% of vaccine-type community acquired pneumonia (CAP), and 75% of invasive vaccine-type pneumococcal disease (IPD) in a large RCT in the Netherlands.[149] Studies of PCV safety and efficacy among IRD patients, mostly measured as antibody response have shown similar results as after PPV vaccination, with in most cases significant antibody responses, however impaired in comparison with healthy individuals. In patients on methotrexate and even more pronounced among those on rituximab, the antibody response after PCV is impaired. [110,150]

Recommendations of pneumococcal vaccinations

Since 2012, the Advisory Committee on Immunization Practices (ACIP) at Centres for Disease Control and Prevention (CDC) recommends pneumococcal vaccination with both PPV23 and PCV13 healthy adults > 65 years of age and to adults ≥ 19 years of age with immunocompromising conditions, functional or anatomic asplenia, cerebrospinal fluid leaks, or cochlear implants. More specifically, the IRD patients are included in the category “Iatrogenic immunosuppression; diseases requiring treatment with immunosuppressive drugs, including long-term systemic corticosteroids and radiation therapy”. Among vaccine naïve patients, one dose of PCV13 should be followed by one dose of PPV23 after at least 8 weeks, and a booster dose of PPV23 5 years later. Patients who earlier received a dose of PPV23 should receive PCV13 after at least 1 year, to avoid hyporesponsiveness.[20,130] The recommendations of The public health agency of Sweden (Folkhälsomyndigheten) and the Swedish Society of Rheumatology (SRF) are in line with CDC ACIP, but with a stronger emphasis of individual and regular assessments of infection risk in each IRD patient, and informed decisions of vaccine distribution in collaboration with the patient. The most recent recommendations from the Task force of European League Against Rheumatism (EULAR) of vaccinations among IRD patients are to be published during 2018. [22,23]

Vaccine effectiveness

The effect of a vaccine is not easily measured. Clinical efficacy with the obvious endpoint of a change in incidence of infections before and after vaccine introduction is influenced by many factors. Since the benefit of most vaccines is well established, it is not always ethically possible to pursue randomized clinical trials including a placebo arm. Diagnostic codes in registries, number of hospitalizations, radiological findings or positive blood cultures of certain microbes or serotyping may all serve as countable events with varying specificity and sensitivity making comparisons between trials challenging. The incidence of infections is also influenced by a number of factors in a society which can change over time as standard of housing, hygiene and level of education. The cohorts need to be large and the studies must go on for a considerable period of time in order to identify significant differences, which poses financial demands.

Antibody response

Prophylactic vaccines as PPV and PCV are expected to induce an immune response with high levels of neutralizing antibodies. Measurements of the specific antibody levels is the commonly used manner to quantify an immunological response and assess the expected level of protection.[151] Antibodies are produced during the first weeks after exposure to an antigen, and if the immune system is triggered in a way that includes T cell activation, some of the plasma cells differentiate into long-life memory B-cells and induce an immunological memory. [143] Hence, the levels of antibodies have become an indirect measure of future protection against infection, and is often called a surrogate marker of vaccine efficacy. The antibody response in *paper II-IV* is evaluated quantitatively and qualitatively with the methods briefly described under "*Patients and methods*".

The antibody response can be expressed in absolute levels (mg/L or μg/ml), or geometric mean levels/concentrations (GML/GMC) when comparing groups. A two-fold or a four-fold increase in the pre-to-post antibody level is sometimes considered a positive antibody response, irrespective of the absolute levels. The fold increase expresses both the immunogenicity of the vaccine and the patient's capacity of reacting to an antigen trigger. Different antibody levels have been proposed as cut points of protective levels after pneumococcal vaccination, differing between 0.15 – 1.6 μg/ml for both infants and adults. [152] Without thorough scientific evidences, partly because of difficulties of comparing results between different laboratories and methods, the consensus of protective levels for adults after pneumococcal vaccination have been settled to 1.0-1.5 μg/ml without further specifying for which serotype.

Out of more than 90 serotypes of pneumococci, the 23 F and the 6B are quantified at our local laboratory. The two chosen serotypes are associated with severe infections and serious clinical outcome among adults and are included in both PPV23 and PCV13. [152,153] In the region of Skåne these serotypes were among the seven most common in pneumococcal blood isolates during 2009-2010.[154]

Both functional and non-functional antibodies are detected with quantitative measures as Enzyme-linked immunosorbent assay (ELISA) and Multiplex fluorescent microsphere immunoassay (MFMI/Luminex). Opsonophagocytic assay (OPA), described below, is a qualitative method to evaluate the percentage of antibodies detected that are functional and contribute to the defence against microbes. [155]

Aims of the present investigation

- to investigate if vaccination with heptavalent conjugated pneumococcal vaccine; PCV7, leads to decreases in the frequency of putative pneumococcal infections among RA and SpA patients in Skåne.
- to explore if pneumococcal serotype specific antibody levels following PCV7 correlate to the frequency of subsequent putative pneumococcal infections among RA and SpA patients.
- to present a legitimate cut off level of serotype specific antibodies (μg/ml) that could predict protection against, or risk of subsequent pneumococcal infection.
- to investigate if belimumab, which inhibits a B cell activating factor, given in addition to standard of care treatment in SLE, affects the antibody response to 13-valent conjugated pneumococcal vaccine.
- to study the impact of methotrexate treatment in RA on the numbers of total and vaccine-specific antibody secreting cells in peripheral blood, and the levels and function of serotype specific antibodies after immunization with 13-valent pneumococcal conjugate vaccine.

Patients and methods

Skåne is the most southern region of Sweden with a population of about 1.3 million inhabitants. The population density is, after the Stockholm region, the highest in the country. [156] There are four rheumatological clinics and a couple of smaller private units in the region monitoring patients with different rheumatological diseases. The majority of the patients have their follow-up at the University clinics in Malmö and Lund.

The ”PREVENAR® vaccination trial”, (registration number NCT 00828997 at clinicaltrials.gov), originally included 505 patients with RA and SpA from the Department of Rheumatology, Skåne University Hospital in Lund and Malmö. The patients all received one dose of heptavalent conjugated pneumococcal vaccine (PCV7) between May 2008 and November 2009. *Paper I and II* include analyses of antibody response before and 4-6 after vaccination from 497 patients, as well as data from the Skåne Healthcare Register (SHR) for ICD-10 codes corresponding pneumococcal infections. The 1988 unvaccinated patients serving as matched controls in *paper I* originates from the whole region of Skåne (*Figure 2*)

The ”VACCIMIL vaccination trial”, (registration number NCT 02240888 at clinicaltrials.gov) was also performed with participation of patients from the Department of Rheumatology, Skåne University Hospital in Lund and Malmö. The cohort included 303 patients with a diversity of inflammatory rheumatic diseases (IRD); RA, systemic lupus erythematosus (SLE), different forms of vasculitis, scleroderma and Sjögrens syndrome as well as 49 healthy individuals serving as controls. All patients and controls received one dose of 13-valent conjugated pneumococcal vaccine. *Paper III and IV* originate from VACCIMIL, focusing on patients with SLE and RA respectively, stratified for different treatments. Several different laboratory analyses on antibody response after vaccination were performed.

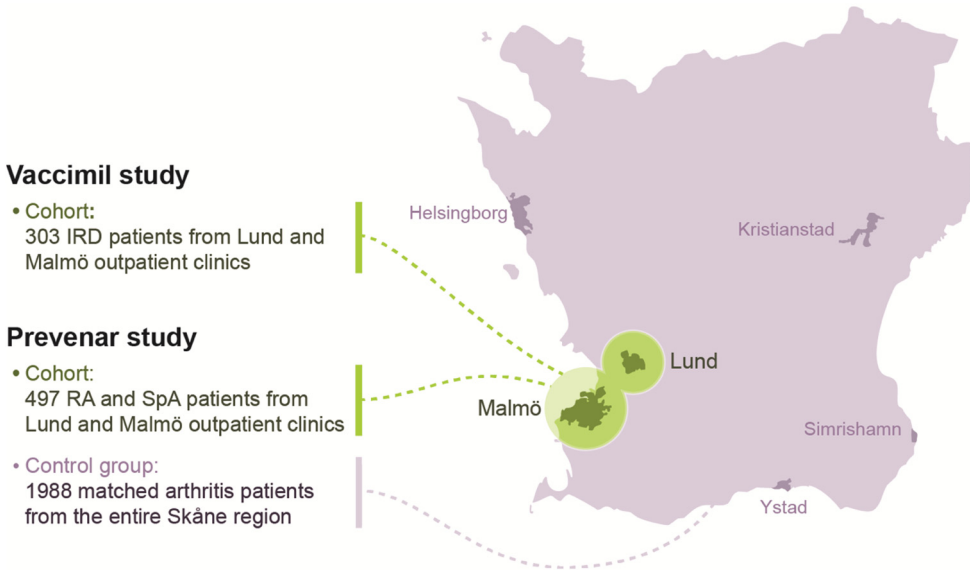

Figure 2.
The Skåne region, including the cities with Reumatology clinics; Lund, Malmö, Kristianstad, Helsingborg, Simrishamn. Both the Prevenar and the Vaccimil cohorts were selected from Lund and Malmö, and the control patients in study I originated from the whole region. Illustration: Jonas Sagard

Skåne Healthcare Register

The Skåne Healthcare Register (SHR) includes information of every individual identification number, all visits at a health care unit, the date of visit and diagnostic codes according to the Swedish version of the International Classification of Diseases (ICD) 10 system.[157]

In *paper I and II*, data from 497 vaccinated arthritis patients with regular follow-up at the Department of Rheumatology, Skåne University Hospital in Lund and Malmö was retrieved from SHR. In addition, 4 anonymous control individuals per patient were identified in the register, with matching of diagnosis (RA or SpA), age (+/- 5 years), sex and residential address in the Skåne region. ICD-10 diagnostic codes corresponding to putative pneumococcal infections registered between 1st of January 2004 and the 31st of December 2012 were retrieved from the register. In *paper I*, the number of infections was compared between the vaccinated known 497 patients, and the 1988 control individuals (arthritis patients from the whole region of Skåne). In *paper II*, the number of infections registered in SHR among the vaccinated 497 patients was compared to the antibody response of each individual.

Enzyme-linked immunosorbent assay (ELISA)

ELISA is a method for identifying and quantifying both antigens and antibodies, first described in 1971 [158] In summary, wells in microtiter-plates are coated with an antigen; in this context the pneumococcal serotype specific polysaccharide.

After incubation overnight, the patient's serum is added, and if present the antibodies bind to their antigens. Finally, a secondary anti-human IgG antibody which has been conjugated to alkaline phosphatase (ALP) and subsequently the substrate p-nitrophenyl phosphate are added, creating a switch in colour when the test is positive. The optical density of each well is measured with a spectrophotometer at 405 nm and related to a reference curve of the antibody concentration (µg/ml).

In order to standardize the measuring of *S. pneumoniae* serotype-specific IgG, WHO published a standard protocol and produced calibration materials and reference standards in the year 2000. [159] This has improved the ability to compare results between laboratories and between serotypes within the same assay.

Opsonophagocytic Assay (OPA)

Functional antibodies should be able to opsonize, or mark out, the antigen they are directed against in a way that leads to uptake of the molecule, virus or bacteria in a phagocytic cell. When analysing pneumococcal antibodies, OPA can be performed in several manners. One or several serotype specific antibodies can be analysed at a time, the bacteria can be alive or killed beforehand, and the result is measured as % of the phagocytic cells with significant uptake of opsonized bacteria, or when analysing living bacteria, as % of the bacteria being killed in the process.[155,160]

In both *paper III and IV* we performed an uptake opsonisation assay on one antibody specificity; 23F. Pneumococci were cultured and killed by glutaraldehyde. They were labelled with fluorescent isothiocyantate (FITC) and then incubated with patient serum, the latter being heat-inactivated in order to eliminate the individual differences of complement factors. Baby rabbit serum was used as external complement source. Neutrophils from healthy donors were incubated with phycoerythrin (PE)-labelled anti-CD66 and thereafter added to the opsonized bacteria. The cells were finally analysed in a flow cytometer, calculating the proportion of cells with uptake of opsonized bacteria, identified as events positive for both FITC and PE.

Enzyme-linked immunospot (ELISPOT)

ELISPOT is an immunoassay developed in Gothenburg, Sweden in the early 1980ies. [161] The analysis enables detection and counting of down to a single cell/100 000 in a cell population, by means of their secreted products; cytokines or antibodies, and was used in *paper IV*. Peripheral blood mononuclear cells (PBMC); B cells, T cells and monocytes, are separated from whole blood via centrifugation. To detect the exact number of serotype specific antibody secreting cells (ASC)/100 000 PBMC, wells with a certain cellulose ester membrane are coated with the antigen (in our case pneumococcal polysaccharide 23F or 6B) and a known amount of the patient's cells are added. If the requested cells are present, antibodies against the antigens are produced and bind to their antigen. After adding a secondary goat antihuman antibody and a substrate, red circular zones (spots), are localized in areas where antibody production has occurred, and can be visually counted in a microscope. Concurrently, other wells are coated with purified goat anti IgA or IgG antibodies. After repeating the same procedure as described above, the total amount of IgA- and or IgG secreting cells can be determined.

Multiplex fluorescent microsphere immunoassay (MFMI / Luminex)

In order to quantify several serotype specific antibody levels simultaneously and to a lower cost than with repeated ELISA, multiplex assays were developed around year 2000.[152] In *paper III*, the analysis was performed at Statens Serum Institut, Copenhagen, Denmark, determining 12 serotype specific antibodies included in both PPV23 and PCV13. The method is based on a flow cytometric methodology. Each antigen, for which the specific antibody level is requested, is attached to a microsphere or bead with an individual fluorescence. The patient's serum is added, followed by the secondary anti-human IgG and a substrate. The microspheres are run through the flow cytometer, enabling quantification of the individual serotype specific antibodies through measurement of fluorescence with different wavelength. [162,163]

Statistical methods

The papers I-IV originated from prospective interventional cohort studies of different sizes. The Prevenar® vaccination study (paper I-II) was originally calculated to include 500 arthritis patients in order to find a difference of 20 % in antibody response between different treatment groups, reaching a statistical power of 80 % and a significance level of 5%. The Vaccimil study (paper III) was planned as a qualitative study with 200 included patients, but continued until a total of 303 patients with different inflammatory rheumatic diseases and healthy controls were included. Paper IV was a pilot study including only 20 RA patients divided in two treatment groups.

In order to describe the demographic characteristics of the cohorts, statistical tests as Chi-square, Mc Nemar's exact, Mann-Whitney U, Kruskal-Wallis or T-Test among others were used when appropriate.

Paper I Focusing on differences in risk of pneumococcal infection depending on vaccination status, we presented the relative risks (RR) of infection after vaccination compared to before vaccination and the ratio between exposed and unexposed groups (RRR). Absolute risk reduction (ARR) is the difference in risk after vaccination between vaccinated and non-vaccinated and the inverted number needed to treat (NNT) was also included. The calculations of ARR and NNT somehow went wrong, and the error was detected after publication. Since the new results led to the same conclusions as the former, but with a stronger protective effect of the vaccine, we did not publish a report of errata. Kaplan-Meier curves were drawn to compare time to first event within and between the groups.

Paper II: Geometric mean levels (GML) of antibodies were obtained with log transformed pre- and post antibody levels. In order to suggest an adequate and clinically relevant protective antibody level, we performed receiver operating characteristic (ROC) curves for each serotype separately. The cut points were chosen on the basis of Youden's index (J statistics) where J = sensitivity + 1-specificy of every point of the ROC curve. The analysis of predictors was carried out with univariate followed by multivariate logistic regression analysis as well as Cox regression analysis.

Paper III. Thanks to the new laboratory method of MFMI (Luminex), multiple continuous variables (antibody levels of 12 serotypes) were measured in every individual before and after vaccination. GML for every serotype were calculated within each treatment group after log transformation. In order to adjust for variation

within the same individual as well as between different individuals in the same treatment group, general linear model for repeated measurements (repeated ANOVA) was performed. Pearson's correlation analyses were executed to internally validate and correlate the three laboratory methods ELISA, OPA and MFMI to each other.

Paper IV GMLs of each of the two included serotypes were compared before and after vaccination (Wilcoxon) and from patients with or without methotrexate treatment (Mann-Whitney U). Proportion of patients with a positive antibody response ($\geq$ 2 fold increase) was compared with Chi-squared test.

Results and Discussion

Paper I:

The risk of pneumococcal infections after immunization with pneumococcal conjugate vaccine compared to non-vaccinated inflammatory arthritis patients

The aims of this study were to compare the risk of all putative pneumococcal infections between RA and SpA patients who did or did not receive pneumococcal conjugated vaccination, as well as to compare the number of infections within the vaccinated as well as the non-vaccinated comparing the time before versus after vaccination.

Table 3.
Demographics, disease and treatment characteristics of the Prevenar cohort.

	All vaccinated (exposed) (n=497)	**RA patients (n=248)**	**SpA patients (n=249)**
Age (years)	55.7 (13.0), 22-88	60.8 (12.4), 24-88	50.6 (11.6), 22-76
Gender, female	63 %	81 %	45 %
Smoking at vaccination	17 %	19 %	16 %
Disease duration, years	14.9 (11.2, 0-48	15.9 (11.5), 0-48	13.8 (10.8), 0-45
DAS28	3.3 (1.24), 0-6.4	3.6 (1.1), 0.6-5.9	2.9 (1.2), 0-6.4
HAQ at vaccination (0-3)	0.7 (0.6), 0-3.0	0.9 (0.7), 0-3.0	0.5 (0.5), 0-2.13
RF at vaccination	-	80 %	-
Anti-CCP at vaccination	-	78 %	-
HLA-B27	-	-	48 %
MTX at vaccination	51 %	69 %	33 %
MTX +anti-TNF at vaccination	32 %	31 %	33 %
Anti-TNF as monotherapy	34 %	35 %	33 %
NSAIDs without other antirheumatic treatment	17 %	0	34 %

248 RA patients and 249 SpA patients shown in *Table 3* were included after a dropout of 8 patients moving from the Skåne region. The SpA patients consisted of a majority of men who were on average 10 years younger than the mainly female RA patients. The estimations of both disease activity and disease duration were somewhat higher among the RA patients.

Almost all events of registered infections, serious and non-serious, were more abundant during the time period after vaccination than before, shown by almost all the dots in *Figure 3* being located to the right of the line. This could be explained by the subjects getting older since no other factors that we know of were altered.

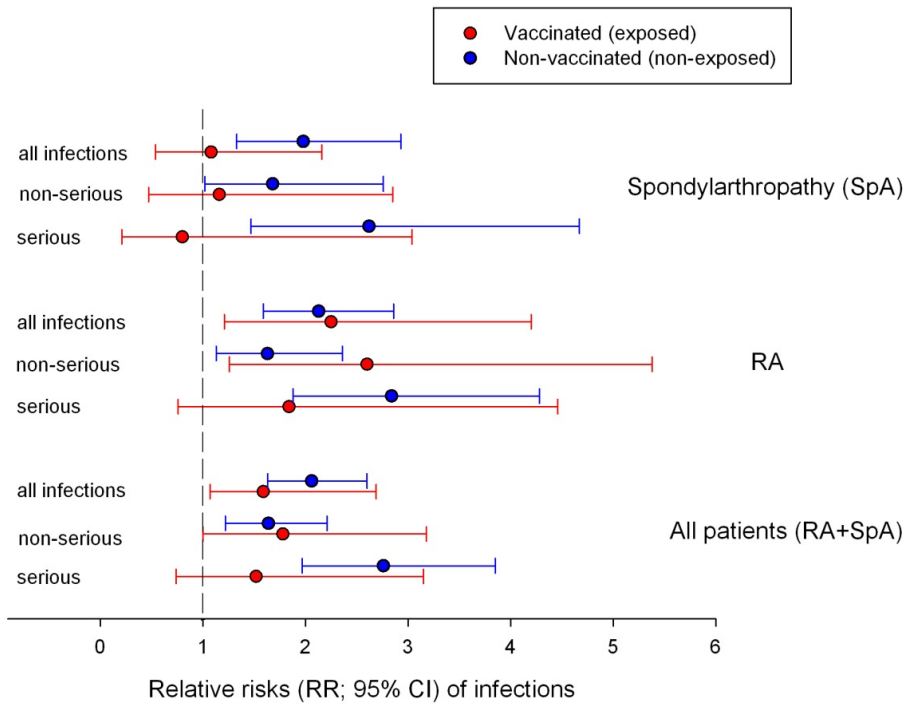

Figure 3.
Relative risks of events in RA, SpA and all patients together

Eighteen serious infections in 15 patients and 27 serious infections in 23 patients occurred before and after vaccination respectively. The majority of these patients had RA. *The main finding* presented in the article (*Table 4*) was a relative risk reduction of 45 % of serious infections (pneumonia, septicaemia, septic arthritis, meningitis), when looking at both RA and SpA together, comparing the time *before and after* vaccination. In both vaccinated and non-vaccinated patients, the number of infections was higher during the years after vaccination than before, but the

increase was greater in the non-vaccinated group. This constitutes the relative risk reduction reported, however not significant.

When looking at only RA patients, there was no absolute risk reduction seen, possibly due to the size of the cohort or a relatively short time period of observation. A probable selection bias might have influenced the result since the vaccinated group of patients were found at the university clinic in Lund with a higher percentage of biologic DMARD use than the average at the time in Skåne. The non-vaccinated cohort however was anonymously identified in the Skåne health care register with surveillance at all different clinics of rheumatology in the region. Even though they were matched for age, sex and arthritic diagnosis, the disease activity, duration of disease or treatment was unknown. PCV was at the time not accessible for the control patients, but the number of control patients who received the PPV23 is unknown.

There was a more obvious protective effect of the vaccine among the younger SpA cohort with a visible absolute risk reduction (ARR) of 2.5% and a very low NNT of 40. However, the absolute number of serious infections were much lower than in the RA group. This group of patients had a somewhat lower disease activity, and a lower percentage of methotrexate use. Comorbidities were not registered and cannot be compared between the subgroups, although it might be reasonable to believe that they were somewhat less abundant in the SpA group. The results support previous findings of how immunogenicity of vaccines can vary and that vaccine efficacy can be negatively influenced and more difficult to measure among the patients who need the preventive measures the most.[110,164]

One could argue that the benefit of the vaccine is lower than expected. Most studies of vaccine efficacy among IRD patients are evaluating humoral response, making it difficult to compare our results with others. In 2015, New England Journal of Medicine published the CAPITA study, an RCT including almost 85 000 healthy adults > 65 years of age. *Bontén et al* showed a significant relative risk reduction of vaccine-type strain community acquired pneumococcal infections, including only events after vaccination. The FDA approval and the inclusion of PCV in CDC recommendations for healthy adults > 65 years of age were based on these results. However, the ARR was very low and the number needed to be vaccinated in order to prevent one infection (NNT) was 13 times higher than in our study.

In *Table 4*, the clinically most relevant endpoint of our study; the serious infections, are included for RA and SpA separately, and all patients together, including corrected calculations of ARR and NNT (see "*Statistical methods*"). In addition to the numbers seen in paper I, the relative risk reduction including only events *after* vaccination, as it is presented in the CAPITA study, is also included. As a comparison, two endpoints from the CAPITA study are shown; first episode of vaccine-type strain community acquired pneumonia (CAP) and first episode of all cause CAP, including non-pneumococcal pneumonia. Relative risk reduction

(RRR), absolute risk reduction (ARR) and number needed to treat or vaccinate (NNT) are shown, subtracted from the number of events published. [149]

Our study has *limitations*. The joint analysis of RA patients and SpA patients, where the latter in turn includes several diagnoses with different characteristics make the interpretation of the results somewhat difficult. The range of both disease activity and treatment is large within the groups, and in the comparative group of non-vaccinated patients these variables are unknown. The identification of events has obvious limitations as they only consist of diagnostic codes without any information of the underlying causal microbe.

Nevertheless, in the context of the population at target and in comparison to studies of PCV efficacy in healthy adults, our study can be said to show a result in favour of the vaccine. The protective effect is more obvious among the younger and presumably healthier SpA patients, while the individuals who need the vaccine the most probably are found among the RA patients where the majority of infections are registered.

Table 4.
Results from paper I with addition of comparative data from the CAPITA study by Bontén et al, New England of Medicine, 2012

	1.	2.	3.	4.	5.	6.	7.
	Relative risk (RR) vaccinated patients; events, **point estimate**, (95% CI)	Relative risk (RR) nonvaccinated patients; events, **point estimate**, (95% CI)	Ratio of RR ; risks, **point estimate**, (95% CI)	Relative risk reduction; 1 - Ratio of RR	Relative risk reduction vaccinated/nonvaccinated patients, including only events ***after*** vaccination (results not included in *paper 1)*	Absolute risk reduction, (ARR) %	Number needed to treat (NNT= 1/ARR)
Spondyloarthritis Serious infection	4/5 **0.80** (0.21-3.04)	41/16 **2.62** (1.47-4.67)	0.80/2.62 **0.31** (0.07-1.31)	**69%**	**61% (-8-86)**	**2.5%**	**40** (22 - 192)
Rheumatoid arthritis Serious infections	23/13 **1.84** (0.76-4.46)	91/33 **2.84** (1.88-4.28)	1.84/2.84 **0.65** (0.24-1.72)	**35%**	**-1% (-56-35)**	-	-
RA and SpA Serious infections	27/18 **1.52** (0.74-3.15)	132/49 **2.76** (1.97-3.85)	1.52/2.76 **0.55** (0.25-1.22)	**45%**	**18.2% (-22.3-45.3)**	**1.2%**	**83** (29 - -94)
*CAPITA first episode of Vaccine type CAP	-	-	-	-	**45.6% (21.8-62.5)**	**0.1%**	**1031** (660 - 2362)
**CAPITA first episode of All cause CAP	-	-	-	-	**5.1% (-5.1-14.2)**	**0.1%**	**1064** (365 – -1162)

1. Relative risk; events after vaccination / events before vaccination among vaccinated patients; RA 248, SpA 249, total 497
2. Relative risk; events after vaccination / events before vaccination among unvaccinated patients; RA 992, SpA 996, total 1988
3. Ratio between the relative risk of the vaccinated patients / the unvaccinated patients, including data from *before and after* vaccination
4. Relative risk reduction among the vaccinated patients including data *before and after* vaccination. 1 – ratio of RR
5. Relative risk reduction among the vaccinated patients including only events *after* vaccination; percentage of vaccinated patients with infections after vaccination *divided* with percentage of unvaccinated patients with infection after vaccination x 100, (95% CI)
6. ARR = percentage of unvaccinated patients with infection after vaccination *minus* percentage of vaccinated patients with infection *after* vaccination x 100
7. Number needed to treat /vaccinate = NNT = 1/ARR

* CAPITA primary endpoint first episode of vaccine type CAP; 49 events among 42 240 vaccinated healthy adults > 65 years of age, 90 events among 42 256 unvaccinated healthy adults > 65 years of age.

** CAPITA other predefined endpoint; first episode of all cause CAP; 747 events among 42 240 healthy adults > 65 years, 787 events among 42 256 unvaccinated healthy adults > 65 years of age.

Paper II:

The association between antibody levels before and after 7-valent pneumococcal conjugate vaccine immunization and subsequent pneumococcal infection in chronic arthritis patients

The *aim* of this study was to assess the association between antibody response and subsequent serious pneumococcal infection in our arthritis cohort (for demographics see *Table 5*). We also wanted to identify the predictors of infection, as well as proposing cut off levels of antibodies (mg/L) to distinguish the likely protected patients from the patients at a larger risk of infection.

Main results: The antibody levels obtained *after* vaccination were significantly lower among the patients who subsequently developed a serious pneumococcal infection. In addition, these patients had borderline significant lower levels of antibodies already before vaccination. This confirms once again the role of antibody response as a surrogate marker of protection. However, the patients who suffered infection *before* vaccination, showed a numerically although not statistically impaired antibody response before vaccination, in contrast to what would be expected of naturally acquired immune response. All patients had significant increases of the antibody levels pre- to postvaccination, confirming the immunogenicity of the vaccine.

The Cox regression model to find predictors of infection included all known variables of the patients, but only higher age, prednisolone at vaccination and higher prednisolone dose (mg/day) remained significant risk factors in the multivariate analysis. The difference in risk of infection between RA and SpA became borderline significant in this setting when adjusting for age and treatment. This confirms what is already known of glucocorticoid use, higher age and risk of infection from previous studies. [106 ,164]

Antibody levels associated with protection against serious infections were identified at 1.29 mg/L for serotype 6B, and 1.01 mg/L for serotype 23F using ROC curves. The cut offs were chosen on the basis of Youden's index (J statistics) where J = sensitivity + 1-specificy of every point of the ROC curve, and on the basis of a clinical discussion where the specificity was considered slightly more important than the sensitivity. Specificity in this context equals the probability of a patient with an antibody concentration above the cut off to be protected against infection. There was however a quite large overlapping of antibody levels between the patients with or without subsequent infection, resulting in a fairly low sensitivity at the chosen cut points. The ROC curve of serotype 23F is shown in *Figure 4*.

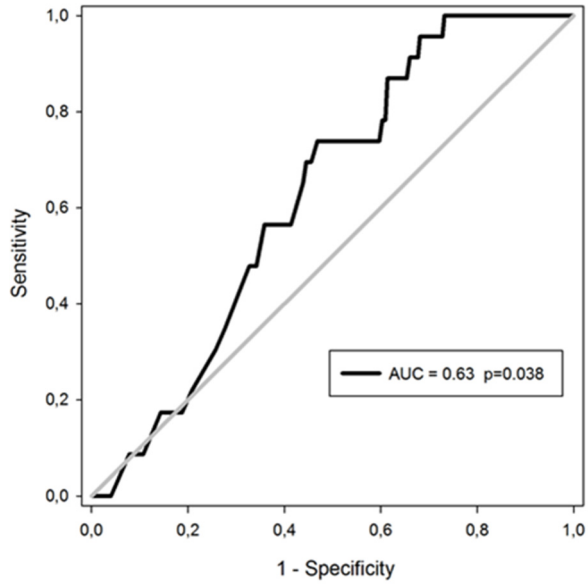

Figure 4.
ROC curve of serotype 23 F.

Table 5 shows an extract of the different postvaccination antibody levels and their associated sensitivity, specificity, positive and negative predictive values. There is no consensus of serological protective levels following pneumococcal vaccination. The immunogenicity varies substantially between serotypes, probably requiring different cut offs. Previous studies have proposed a range of levels from 0.15 mg/L to 1.6 mg/L for serotype specific antibodies following both polysaccharide and conjugated pneumococcal vaccine in both children and adults. [152] Our results are in line with antibody levels of 1 mg/L used in studies of a polysaccharide vaccine against H. Influenzae b (Hib).[165] but should not be considered a strict proposal of clinically usable cut points, but rather an indication of arbitrary protective levels of these particular serotype specific antibodies; 23F and 6B.

Table 5.
Different postvaccination antibody levels with associated sensitivity, specificity, positive- and negative predictive values.

Serotype 6B				
Postvaccination antibody levels (cut off)	Specificity (%)	Sensitivity (%)	Positive predictive value% (95%CI)	Negative predictive value %(95%CI)
0.69	85.1	26.1	7.79% (2.93-16.20	95.95% (93.60-97.62
1.02	79.1	30.4	7.00% (2.87-13.9)	95.97% (93.5-97.7)
1.29	**75.1**	**39.1**	**7.03% (3.3-12.9)**	**96.2%(93.7-97.9)**
1.31	74.9	39.1	7.09% (3.3-13.03)	96.2%(93.7-97.9)
1.45	73.0	39.1	6.62% (3.08-12.19	96.12% (93.58-97.86
Serotype 23F				
Postvaccination antibody levels (cut off)	Specificity (%)	Sensitivity (%)	Positive predictive value %(95%CI)	Negative predictive value %(95%CI)
0.71	80.0	21.7	4.85% (1.61-10.97	95.43% (92.87-97.27
0.88	75.3	26.1	5.17% (1.93-10.92	95.54% (92.95-97.38
1.01	**73.0**	**34.8**	**5.88%(2.6-11.3)**	**95.8% (93.2-97.7)**
1.10	71.3	34.8	5.56%(2.4-10.7)	95.8% (93.1-97.6)
1.11	71.1	39.1	6.47%(3.01-11.94	96.09 (93.52-97.84

This study has *limitations*. In spite of a borderline significant difference in antibody response between RA and SpA patients the rest of the analysis was done of the whole group of arthritis patients together since the number of events in general was low. The endpoint of serious pneumococcal infection originated from diagnostic codes with several potential sources of error, see paper I. The patients were vaccinated with an heptavalent conjugated pneumococcal vaccine, however the humoral response was evaluated measuring levels of only two of the serotype specific antibodies.

Paper III:

Treatment with belimumab in systemic lupus erythematosus does not impair antibody response to 13-valent pneumococcal conjugate vaccine

The *aim* of the third study was to assess differences in antibody response after 13-valent PCV vaccination among SLE patients with or without belimumab in addition to standard of care therapy. All 47 SLE patients from the VACCIMIL (see "Methods and patients" page 38) cohort were included as well as 21 healthy controls (HC). The patients were divided into 5 different treatment groups and HC served as group 6. Demographics and disease characteristics of the cohort are seen in *Table 6*.

Table 6.
Demographics and disease characteristics SLE patients and HC.

	SLE No DMARD (n=7)	AZA only or DMARD other than HCQ (n=9)	AZA + HCQ (n=10)	HCQ only (n=10)	Belimum ab + standard treatment (n=11)	Controls
Mean Age, yrs (SD)	63.3 (7.0)	58.3 (4.2)	56.3 (5.3)	44.1 (3.5)	37.7 (3.9)	43.6 (3.0)
Women, n (%)	7 (100)	9 (100)	9 (90)	9 (90)	10 (91)	18 (85.7)
Mean Disease duration (SD)	20.1 (4.7)	21.9 (4.9)	22.1 (6.8)	11.4 (2.8)	7.5 (2.4)	-
Mean SLEDAI score (SD)	0,86 (0.59)	2.4 (0.99)	1.5 (0.62)	1.4 (0.79)	3.0 (0.67)	-
Prednisolone at vacc, n (%)	2 (29)	9 (100)	8 (80)	3 (30)	10 (91)	-
ANA positivity (%)	100	100	100	100	100	-
Anti-ds-DNA positivity (%)	0	56	50	40	64	-

Antibody levels of 12 out of 13 serotypes were significantly elevated among both SLE patients and healthy controls after vaccination compared to before vaccination, but the levels were lower among SLE patients as a group compared to HC. This finding is in line with earlier observations of elevated risks of infection among SLE patients irrespective of ongoing treatment.[5,10,166]

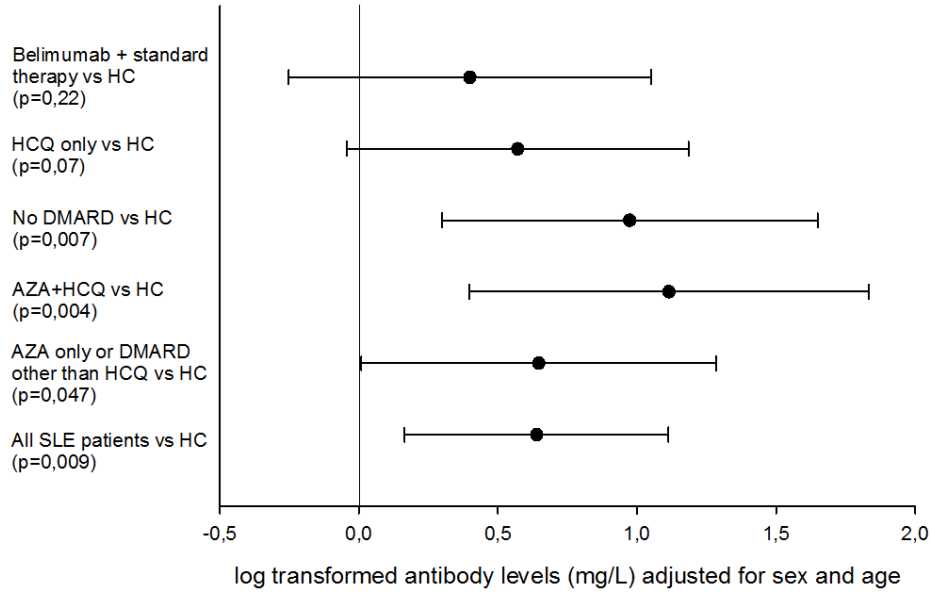

Figure 5.
Comparison of fold increase between every treatment group of SLE patients and healthy controls (HC), and at the bottom HC versus all SLE patients together.

Main results: Our hypothesis, that treatment with belimumab in addition to SOC would impair antibody response could not be confirmed. Comparing the group on belimumab treatment to every other treatment group, adjusted for age and sex, there was no significant difference in either post vaccination antibody levels, or in fold increase. When instead comparing the fold increase of the healthy controls to every treatment group of SLE (*Figure 5*), there were no differences between the belimumab group and HC nor between patients with only HCQ treatment and HC.

Similar results were later published by *Chatham et al* 2017, comparing antibody response after PPV23 among SLE patients who were immunized before starting belimumab or during concurrent treatment. [167] Similarly, in a substudy to the RCT BLISS-76, pre-existing antibodies to 12 serotypes of pneumococci were analysed at baseline and after a year of belimumab treatment, finding no differences between the groups that would imply impairment of memory B cells during belimumab treatment. In addition, 7 patients in total received PCV13 during that trial, with positive antibody response to most serotypes. [168]

In our study, the patients on belimumab in addition to SOC were the youngest of the SLE groups, and none of them had experienced SLE nephritis. Their disease activity was about the same as the other groups at the time of vaccination, but with

higher dosages of prednisolone than the others. When looking at all SLE patients, higher age was significantly associated with an impaired antibody response, but when excluding patients > 60 years of age from the analysis, this correlation was no longer significant. Hence, in our point of view, the absence of the expected impairment in antibody response among the belimumab treated group of SLE patients could not be explained by demographic or disease characteristics.

Antibody response can, as previously described, be measured in different ways. For the first time in our group, we used the MFMI/Luminex method in order to enable analysis of multiple serotype specific antibodies, and also the opsonophagocytic assay/ OPA to estimate the functionality of the antibodies. There was a significant correlation between the results from these two methods, as well as between golden standard ELISA and OPA, and ELISA and MFMI.

The small size of the cohort was the obvious *limitation* of this study. Since the smallest treatment group (without DMARD treatment) comprised only 7 patients, we could not include multiple covariates in the analysis such as adjustment for different ongoing treatments. In the process of peer review we were asked to reorganize the patients into larger groups to address this issue; 1) SLE without DMARDs or only HCQ, 2) AZA and HCQ or only AZA or other DMARD, 3) belimumab plus standard treatment, and 4) healthy controls. The main results previously declared could be confirmed, but further inclusion of covariates was still not possible. The mean treatment duration of belimumab in our study was 9 months, and only half of the 11 individuals on belimumab had had the treatment for more than 6 months. Results from the large phase III trials BLISS -52 and BLISS -76 show results pointing at a biologic effect of the drug already after 3 months, however studies of antibody response after longer periods of treatment are lacking.

Paper IV:

Methotrexate reduces vaccine-specific immunoglobulin levels but not numbers of circulating antibody-producing B cells in rheumatoid arthritis after vaccination with a conjugate pneumococcal vaccine

The *aim* of the fourth study was to further map out the impact of MTX on B cell function among RA patients before and after conjugated pneumococcal vaccination. The levels of IgA- and IgG serotype 23F and 6B specific antibodies, the functionality of the antibodies and the number of total and serotype specific antibody secreting cells were compared between RA patients with and without MTX treatment.

There was no difference between the groups in terms of age, gender, or percentage of RF and ACPA. The group without MTX consisted of newly diagnosed RA patients with significantly higher disease activity but a shorter disease duration than the MTX treated group, see *Table 7.*

Table 7.
Demographic, disease and treatment characteristics of the study population

	Rheumatoid arthritis patients		
	Receiving MTX (n=10)	**No DMARD (n=10)**	***P* value**
Age; years, median (range)	67.4 (39.1-78.6)	67.3 (38.6-86.7)	0.912[a]
Sex (% female)	70	80	0.606[b]
Disease duration at vaccination; years, median (range)	8 (1-39)	0 (0-12)	**0.035**[a]
ACPA positive (%)	70	50	0.317[b]
RF positive (%)	80	80	1[b]
SJC at vaccination (0-28) median (range)	0 (0-6)	6.0 (0-17)	**0.011**[a]
TJC at vaccination (0-28), median (range)	0 (0-6)	3.5 (0-15)	**0.007**[a]
Erosive disease (%)	30	40	0.639[b]
CRP at vaccination; mg/L, median (range)	3.3 (0-11)	5.8 (0-48)	0.436[b]
ESR at vaccination; mm/hour, median (range)	15.5 (5-42)	29 (8-46)	**0.028**[a]
HAQ at vaccination (0-3), median (range)	0.3 (0-0.55)	0.6 (0.1-1.9)	**0.030**[a]
MTX dose; mg/week, median (range)	20 (12.5-25)	-----	

We hypothesised that the previously documented decrease in antibody levels seen among arthritis patients on MTX treatment before and after different vaccinations would be associated with a corresponding and measurable decrease in the number of antibody secreting cells or plasmablasts. This could not be confirmed.

Main results. There were no significant differences in total numbers of IgA and IgG producing plasmablasts between RA patients with or without MTX 6 days after conjugated pneumococcal vaccination, nor any difference in serotype specific plasmablasts for 23F and 6B, (*table 8).*

Table 8.
Postvaccination number of IgG and IgA total and specific antibody-secreting cells /100 000 peripheral blood mononuclear cells (PBMC).

	Rheumatoid arthritis patients		
Number of Ig-producing cells/1x10^5 PBMCs	**Receiving MTX (n=10)**	**No DMARD (n=10)**	***P* value[b]**
Total IgG	195 (10-840)[a]	170 (70-560)	0.78
Anti-6B IgG	1.25 (0-11.5)	1.75 (0-5)	0.68
Anti-23F IgG	2.3 (0-34.5)	1.5 (0-4.5)	0.28
Total IgA	65 (5-800)	100 (20-650)	0.38
Anti-6B IgA	0 (0-2.5)	0 (0-2.1)	0.61
Anti-23F IgA	0 (0-1)	0.5 (0-5.5)	0.20
[a] Median (range) [b] Mann-Whitney U test			

Both quantity and quality of the serotype specific antibodies were however significantly impaired in the MTX group. Already before vaccination, the group with MTX had numerically but not statistically lower antibody levels of serotype 23F and 6B, (*table 9*). Only one out of 10 patients on MTX had a positive antibody response (≥ 2 fold increase) to both serotypes pre- to postvaccination, whereas four patients without treatment were responders.

The geometric mean antibody levels (GML) of the MTX treated group was significantly increased in one out of two serotype specific antibodies; 6B, but when scrutinizing the data only one single patient had a major response, *(figure 6)*. There was no significant increase or decrease in antibody levels of 23F pre- to postvaccination in the MTX group. The untreated group showed significant increases in GML to both serotypes. On a group level, both patients with and without MTX reached putatively protective levels (≥ 1mg/L)[152] both before and after vaccination (*table 9*)

Table 9.
Pre- and postvaccination geometric mean levels of vaccine-specific anti-6B and anti-23F IgG in serum.

Rheumatoid arthritis patients		
	Receiving MTX (n=10)	**No DMARD (n=10)**
Pre anti-6B IgG; g/L (95% CI)[a]	1.3 (0.5-32)	2.5 (0.7-8.3)
Post anti-6B IgG; g/L (95% CI)	2.1 (0.7-6.4)	5.7 (2.1-15.4)
Pre anti-23F IgG; g/L (95% CI)	1.0 (0.3-4.1)	2.4 (1.2-4.7)
Post anti-23F IgG; g/L (95% CI)	1.7 (0.6-4.4)	10.1 (5.2-19.5)[b]
[a]GML (geometric mean levels); [b]p=0.007 (Mann-Whitney U test) MTX=Methotrexate, DMARD=disease modifying anti-rheumatic drugs		

The functionality of the antibodies was analysed with opsonophagocytic assay (OPA) of serotype 23F in 4 randomly chosen patients from each treatment group. Only 1 out of 4 MTX treated patients showed an increase in opsonophagocytic activity, i.e. proportion of polymorphonuclear cells with a significant uptake of bacteria. ¾ patients without MTX showed an increased OPA, *figure 7*.

In summary, MTX treatment in dosages used in arthritis patients (≤ 25 mg/week) appears to hamper the B cell function as shown by antibody response with both quantitative and qualitative measures, but does not seem to impair the particular differential B cell stage examined in this study; the antibody secreting plasmablasts. The antibody levels in the MTX treated group in our study were decreased (however not significantly) already before vaccination which could be pointing at a possible impact on the memory B cell compartment. Previous studies of patients with juvenile idiopathic arthritis (JIA) treated with MTX have on the other hand shown relative and absolute reductions in the earlier stages of B cell differentiation, the transitional B cells.[113].

MTX is an anchor drug in the treatment of arthritic disease making its immunosuppressive mode of action an important subject to further investigate, even though the numbers of serious infections among MTX treated patients does not seem to be a large clinical problem.

This study has *limitations*. The number of patients was low with only 10 patients in each group. Only 2 out of 13 vaccine serotypes (23F and 6B) were analysed with ELISA, and OPA was performed in only 8 patients of one single serotype; 23F. However, the results were consistent with previous findings of reduced antibody response in both absolute levels and function among patients on MTX in antirheumatic doses. [110-112]

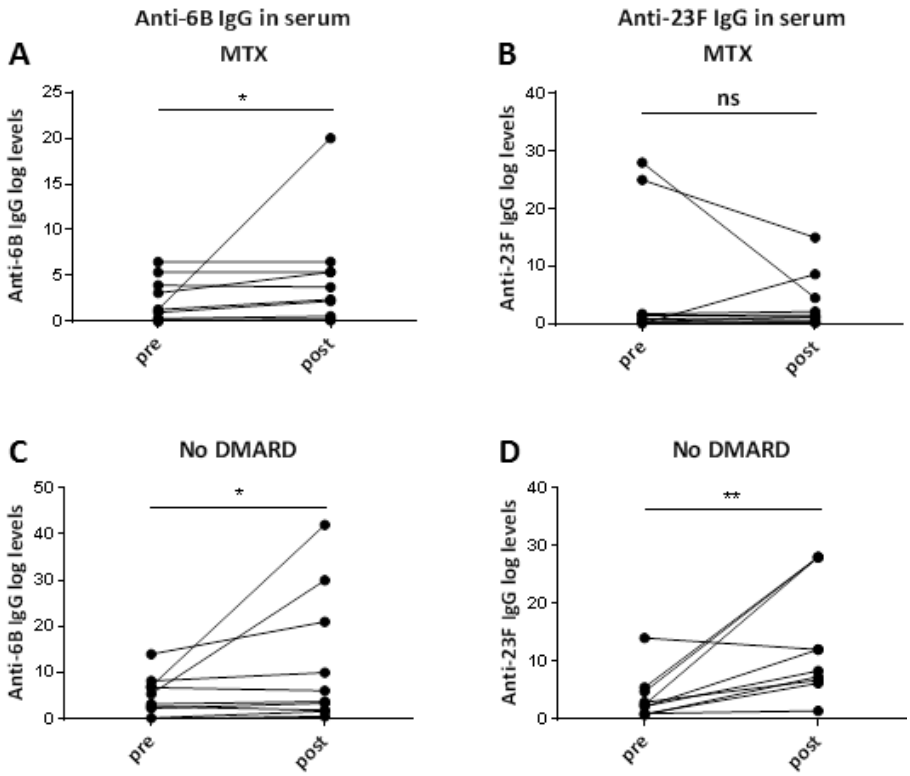

Figure 6.
Pre- and postvaccination antibody levels for serotype 6B and 23F before and 4-6 weeks after vaccination with PCV13 in RA patients on MTX (A and B) and RA without DMARD (C and D).*p<0.05 and **p<0.01 (Wilcoxon test).

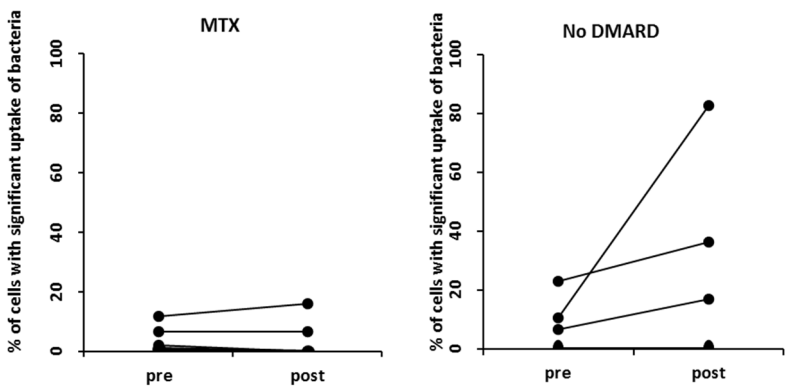

Figure 7.
Opsonophagocytic activity of pneumococcal serotype 23F in 4 patients with ongoing MTX treatment and 4 patients without DMARD treatment

Conclusions

- Heptavalent pneumococcal conjugated vaccine in RA and SpA patients induces a relative risk reduction of subsequent putative serious pneumococcal infections up to 5 years after the immunization.
- There is a significant correlation between humoral response of serotype 23F and 6B and subsequent serious putative pneumococcal infections among RA and SpA patients.
- A significant humoral response to the vaccination with antibody levels about 1-1.3 mg/L of serotypes 23F and 6B is associated with subsequent reduced risk of serious pneumococcal infection among arthritis patients.
- The risk of serious pneumococcal infection increases with higher age and glucocorticoid use among arthritis patients.
- Belimumab given to SLE patients in addition to standard treatment during about 9 months does not further impair the antibody response after 13-valent pneumococcal vaccination.
- The results of the three laboratory methods used to evaluate humoral response among SLE patients after vaccination; ELISA, MFMI and OPA correlate to each other.
- The impairment in humoral response and functionality of antibodies seen in MTX treated RA patients is not associated with decreased numbers of antibody secreting plasmablasts, thus probably not due to reduced activation of B cells in lymphoid tissue.

Future perspectives

With a growing global population and limited resources, preventive measures in every field of the health care system will continue to be of great importance. Since the late 18th century when the smallpox vaccine was developed by dr Edward Jenner, immunization has been a large chapter of preventive medicine. The underlying infectious agents that cause the diseases continue their evolution by developing and altering virulence factors in order to spread. The growing threat of bacteria resistant to antibiotics emphasizes the importance of prevention even further, especially in risk individuals such as patients with IRD.

Out of more than 90 capsular serotypes of pneumococci, about 30 have shown significant invasiveness. The knowledge of newly discovered serotypes, capsular subtypes and microevolvement through mutations from one capsular subtype to another when already in the host tissues, is constantly growing. [129] After introduction of the first PCV in the year 2000, the seven serotypes initially included have decreased significantly in both colonization- and disease prevalence in several geographic settings. [129,132,169] More vaccine serotypes have thereafter been added, and the efficacy of 10-, 13- and 15-valent PCV has repeatedly been documented among several risk groups. [170-172] However, disease associated with non-vaccine type (NVT) serotypes has concurrently been increasing, sometimes to an extent that the overall benefit of the vaccine introduction was nullified. [173] This phenomenon is called serotype replacement or serotype shift, and the ongoing efforts to develop vaccines with more and more serotypes have been compared to a race with nature.[129,132] A new generation of pneumococcal vaccines inducing a serotype-independent immunity are under development, including the use of subcapsular proteins as antigen, and whole cell vaccines. [174] [130,175]

The number and modes of action of new antirheumatic treatments are continuously growing, already including both monoclonal antibodies against extracellular cytokines, and intracellular agents e.g. JAK inhibitors. For every new substance licensed, post marketing evaluations of the risk of infections will be needed. Serotype specific antibodies are still considered the primary host defence against pneumococcal infections, opsonizing the bacteria leading to complement-dependent phagocytosis.[129,174] As long as the vaccines are based on the capsular polysaccharides, the measurement of serotype specific antibodies will probably remain the way to evaluate whether a patient is protected against infection or not.

Since the capsular serotypes vary extensively in terms of immunogenicity, virulence and pathogenic features, as well as in significance between different geographic settings, the development and access to methods enabling analyses of multiple serotypes simultaneously, both quantitatively and qualitatively, will be needed. Repeated studies and approximations of protective levels of a few common serotypes will be useful in order to establish reference cut off levels.

Further exploration of the treatment impact on specific immune cells and their subtypes remain. In paper IV we performed a pilot study counting serotype specific and total antibody producing B cells; plasmablasts. MTX treated RA patients did not have lower levels of cells than untreated patients, and hence no larger study was set up. However, the effect of MTX on other differential stages of B- and T cells is still to a large extent unexplored. One possible way forward would be to analyse regulatory T cells involved in the activation of humoral response.

Our results, among others, are pointing at a considerable heterogenicity among IRD patients in terms of risk of infection. Not only the diagnosis or treatment or age predict the risk, but a combination of these and other factors. The Advisory Committee of immunization practices (ACIP) of the Centers for Disease Control and prevention (CDC), the public health agency of Sweden and the Swedish Society of Rheumatology are all recommending the same schedule of pneumococcal vaccines to patients with IRD; starting with the PCV13 followed by PPV23 at least 8 weeks later. [20,22,23] The Swedish recommendations are however more clearly emphasizing the importance of an individual repeated assessment before vaccination, in comparison to the ACIP version. Development of a user-friendly tool or index to select which IRD patients have the higher risk of infections and are likely to benefit from vaccinations, will be one important future task. There are already a few such scoring systems in limited use, but only including RA patients.[6,61,62]

Surveys have shown suboptimal pneumococcal vaccine uptake among IRD patients around the world, ranging from zero to about 60% between countries. [139] The most important factors affecting the vaccine uptake seem to be the attitude, knowledge and awareness of the treating physicians, indicating a potential improvement of how current recommendations are communicated.[141]

The number of available vaccines is growing. However, it is not necessarily reasonable to provide everyone with every kind. The virulence factors of the infectious agents are evolving while the development of new vaccines constantly is one step behind. The ethically and financially reasonable number of individuals needed to be vaccinated in order to prevent one infection will and should vary between settings, groups at target and infectious agents. Future vaccine research will need to further address both aspects of vaccine reluctance and scepticism

among those who would benefit, as well as the possible thrive among producing companies to expand vaccine recommendations.

In summary, the future tasks in the field of pneumococcal vaccination among IRD patients will be to 1) continuously map out the impact of new antirheumatic treatments on the risk of infection, 2) further develop and agree on accessible analytic measures and cut offs to identify patients at risk, 3) to develop a user-friendly tool or scoring system in order to identify patients at high risk of infection and thus most likely to benefit from vaccination and 4) to prove that the current vaccination strategy protects against pneumococcal infections among the targeted population.

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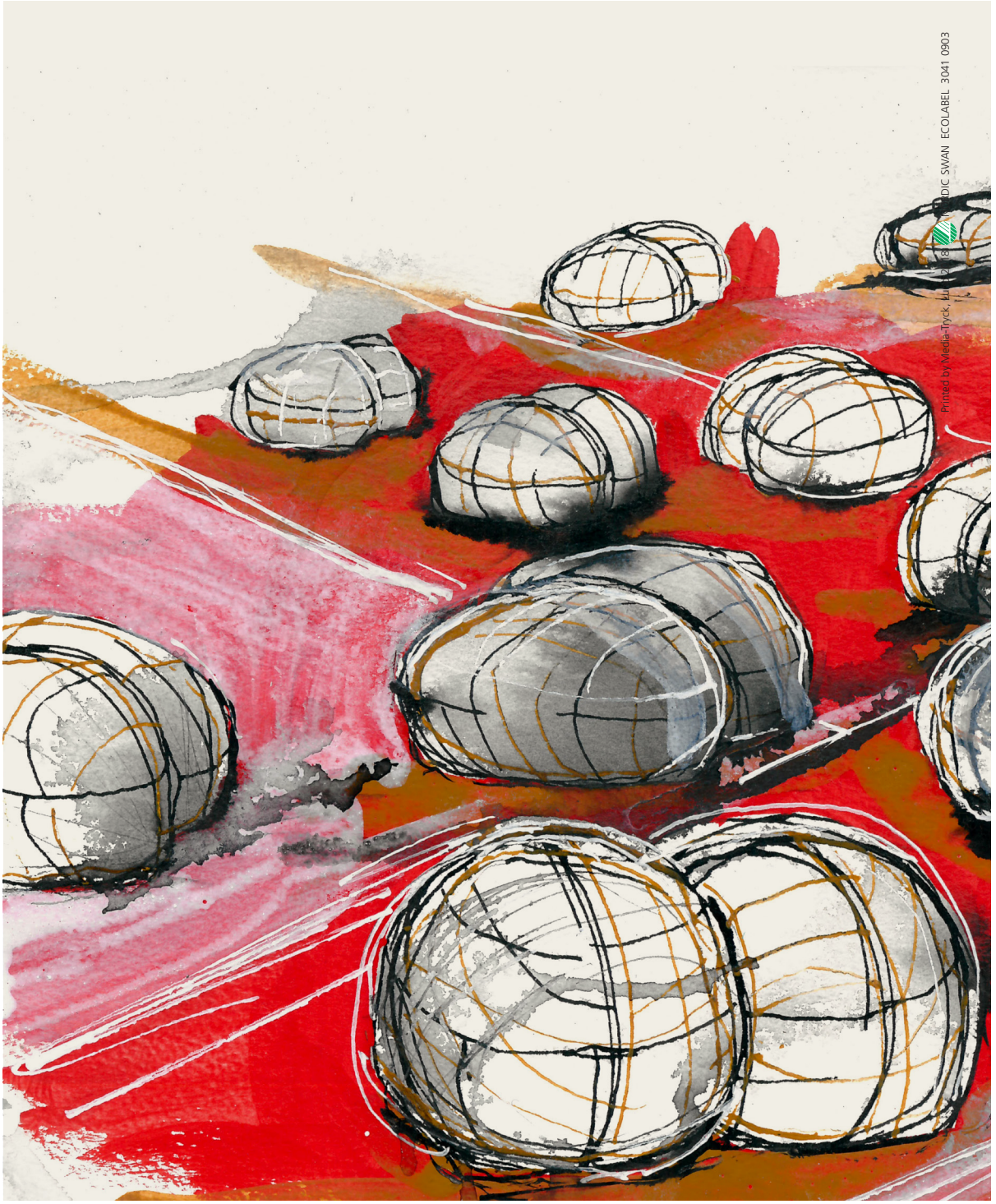

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